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A MODULE FOR CHEMICAL CONTROL OF MOSQUITO VECTORS

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The booklet "A Module for Chemical Control of Mosquito Vectors" has been prepared by the VCRC for training purposes only. The information contained therein has been culled from several publications which are listed below.

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2. L.K.H. Goma (1966). The Mosquito.

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4. Equipment for Vector Control (1974) WHO publication.

5. Safe Use of Pesticides (1985). WHO Technical Report Series, 720.

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A Module for Chemical Control of Mosquito Vectors

CONTENTS

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1. Mosquito Borne Diseases.

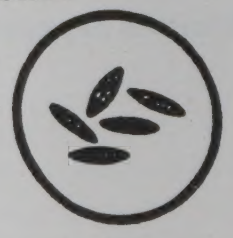
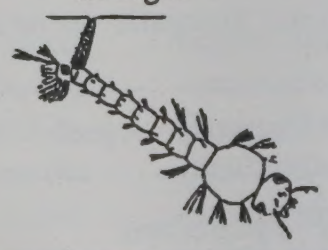
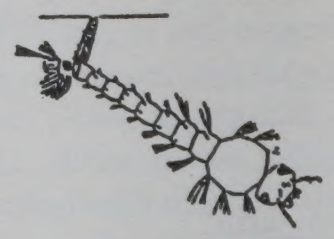
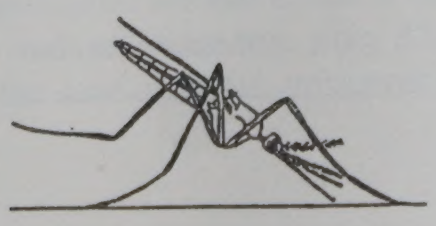
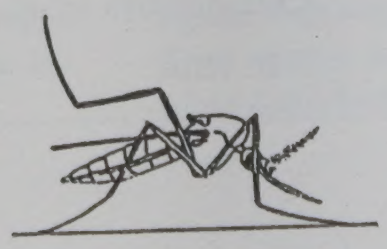
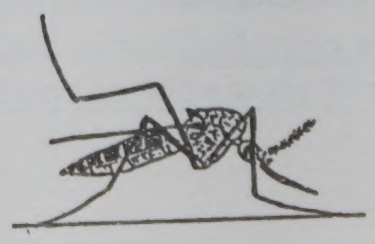
Mosquitoes are the most important single group of insects in terms of public health significance. These remarkably adaptable insects continue to successfully coexist with man, feeding on him and his domesticated animals. Besides the colossal blood loss they are also capable of transmitting many diseases like filariasis, malaria, yellow fever, Japanese encephalitis, dengue etc.

It is estimated that approximately 40 million people in India suffer from mosquito borne diseases annually.

Mosquitoes belong to the family Culicidae of the order Diptera (i.e., the two-winged flies). They are small, long-legged, two-winged insects; the adults differ from other flies in having an elongated mouth or proboscis and scales on the wing veins and wing margins.

There are over 3000 mosquito species belonging to 34 genera in the world. Of these, only about 300 transmit human and animal diseases. Mosquitoes belonging to three genera, Culex, Anopheles and Aedes are known to transmit the major mosquito-borne diseases. The characteristic appearance of the various stages of the three genera is given in Table 1.1.

TABLE 1.1. DIFFERENTIATION OF *CULEX; ANOPHELES* AND *AEDES*

STAGE	ANOPHELES	CULEX	AEDES
EGG	laid singly, possess floats 	laid in groups called rafts, floats absent 	laid singly, floats absent. 
LARVA	The body and the head lies parallel to the surface of water. 	The body and head hangs downwards at an angle. 	The body and head hangs downwards from the surface of water. 
ADULT	The body is inclined at an angle of 45° to the surface except *A. culicifacies*	Body lies parallel to the surface.	Body lies parallel to the surface.
	Greyish hairy body with spotted wings except *A. aitkeni* & A. culiciformis 	Body greyish but wings not spotted. 	Black and white bands on the body and legs. 

About 50 species of anopheline mosquitoes are found in India. Of these, *Anopheles culicifacies, Anophęles fluviatilis, Anopheles stephensi, Anopheles sundaicus, Anopheles minimus, Anopheles balabacensis, Anopheles philippinensis, Anopheles annularis, Anopheles varuna,* and *Anopheles jeyporiensis* are important malaria vectors. Other anophelines transmitting malaria are of local importance.

The incidence of Malaria in India has reached a plateau at about 2 million cases annually.

The Culex fauna consists of 57 species of which only few species transmit human diseases. Culex quinquefasciatus transmits filaria caused by the nematode Wuchereria bancrofti.

Latest official estimates in India showed that 304 million people are reported to be exposed to the risk of infection with an estimated 22 million microfilarial carriers and 16 million chronic filariasis cases. It is thus said that every third person in India runs the risk of filarial infection.

Culex tritaeniorhynchus and *Culex vishnui* group of mosquitoes transmit Japanese encephalitis.

The epidemic outbreaks of JE continue to occur year after year and thousands of young children die of this dreadful disease.

The genus Mansonia is represented by seven species. Of these *Mansonia annulifera* and *Mansonia indica* transmit *Brugia malayi.* Malayan filariasis though localized to some pocket, the deformity caused by this parasite is much more severe than that due to bancrofti.

The genus *Aedes* is represented by several species in India. Only *Ae. aegypti* is the vector of dengue hemorrhagic fever.

Approximately about 1 million people in India are said to suffer from these diseases annually.

Some of the diseases are transmitted by more than one species of mosquito. It is also important to realize that a species which appears to be an excellent vector in one place may be of little or no importance in another. Some of the important mosquito borne diseases in India and the vectors transmitting such diseases are presented in the Table 1.2.

TABLE 1.2. *Some Mosquito borne diseases and vectors*

Disease	Causative Agent	Recognized vectors
1. Malaria*	*P. falciparum* *P. vivax* *P. malariae* *P. ovale*	*Anopheles culicifacies,* *An. fluviatilis, An. maculatus* *An. minimus, An. sundaicus and* *An. stephensi*
2. Lymphatic Filariasis:		
Bancroftian	*W. bancrofti*	*Cx quinquefasciatus*
Malayan	*B. malayi*	*M. annulifera, M. uniformis*
3. Break bone fever	Dengue virus	*Ae. aegypti*
Chikungunya	Chikungunya virus	*Ae. aegypti*
4. Encephalitis	J. E. virus	*Cx. tritaeniorhynchus*

2. Life Cycle

Life cycle of mosquitoes is complex in nature since a part of it is spent in the aquatic environment and part in a terrestrial environment. The mosquito life cycle (Fig 2.1) consists of four stages namely EGG, LARVA, PUPA and the ADULT. Except for the adult stage, all the other stages develop in water (aquatic stages).

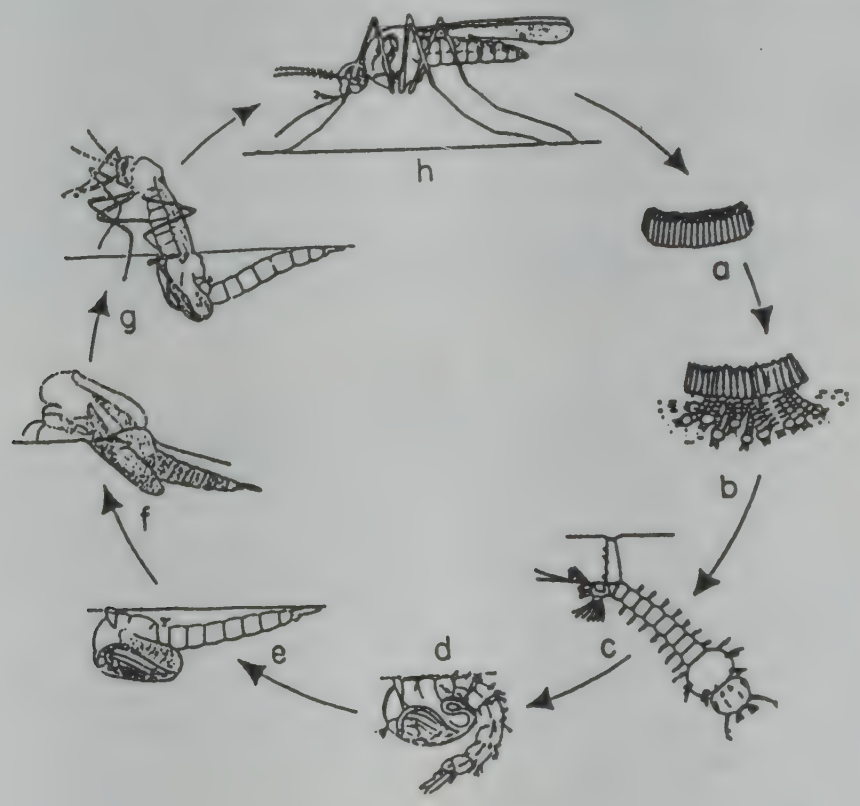


Fig 2.1

2.1. EGG : The eggs are generally laid on the water surface (as in the case of *Culex* and *Anopheles*) or on damp surfaces near water sources (as in *Aedes* and *Armigeres*). While most species deposit their eggs on the water surface, few of them lay eggs on the upper surface of floating vegetation and large number oviposit at varying distances from the water's edge amongst leaf litter, mud and debris or on the walls of man-made containers, plants, tree-holes and bamboo etc. There is a misconception that trees and bushes are responsible for mosquito breeding but they only provide ideal resting places for outdoor resting mosquitoes. However water stagnation in tree-holes during rainy season is found to be an ideal breeding ground for *Aedes*. Eggs of mosquito are laid either in the form of rafts as in the case of *Culex* spp. (Fig 2.1a) or individually as in the case of *Aedes, Armigeres* and Anopheles species. These eggs float on the water surface and number varies from species to species. These eggs hatch into 1st instar larvae (Fig 2.1b) within 24 hours in tropical conditions.

2.2. LARVA : The larva emerges out of the egg with the help of an egg breaker on its head. The larva then begins to feed and grow bigger and bigger. Mosquito larvae are very active swimmers and they feed on organic particles, dead plant substances and other planktonic substances. When grown sufficiently, the outer body cover is periodically discarded by a process called moulting and the subsequent stage is the next instar. The 1st instar larva undergoes three moultings to become the fourth instar larvae (Fig 2.1c) which moults into the next stage of the life cycle called pupa.

2.3. PUPA : The pupae are 'Comma shaped' with a flexible abdomen for swimming and a pair of trumpet like structure on the dorsal surface for breathing (Fig 2.1d). At this stage they do not feed and they are lighter than water and float at the surface of the water. They appear as round immobile floating bodies and when disturbed they are as active as the larval stage

and moves about in water by the jerking of its body. In about 2 - 3 days the pupa splits and the adult emerges (Fig 2.1e & f). Developmental period from egg to adult varies from species to species and is also temperature dependent, though in tropical conditions it is about 7 - 10 days.

2.4. ADULT : Equal numbers of male and female adult mosquitoes continuously emerge from the breeding sites . The emerged adults (Fig 2.1g) rest for some time near the breeding site and later flies off to suitable resting sites till its wings and legs are well stretched and body hardens. It is the female mosquito that bites man and sucks the blood for the development and maturation of its ovaries. During the act of feeding blood, these mosquitoes transmit parasites and cause diseases such as Malaria, Filaria, Japanese encephalitis and Dengue. In general first blood meal is taken on the third day after emergence and subsequently every second day. On an average adult mosquito survives for 15 to 30 days in tropical climate. The male mosquito on the otherhand feeds only on nectar and it mates with the females which produce fertilised eggs.

The orientation of mosquito swarms over contrasting and sharply defined points has often been observed in nature. The swarming and mating habits of mosquitoes depend on various factors like light, sound, wind, temperature and humidity. There is a fairly extensive literature on the mating habits of mosquitoes. It is observed that some species readily mate in small cages and are termed as "stenogamous" whereas those require outdoor conditions for mating are termed as "eurygamous". This is based on the assumption that the prime difference in mating habits among mosquito species is space requirement.

3. Morphology

The body of the adult mosquito is divisible into three distinct regions: the head, the thorax and the abdomen. (Fig 3.1)

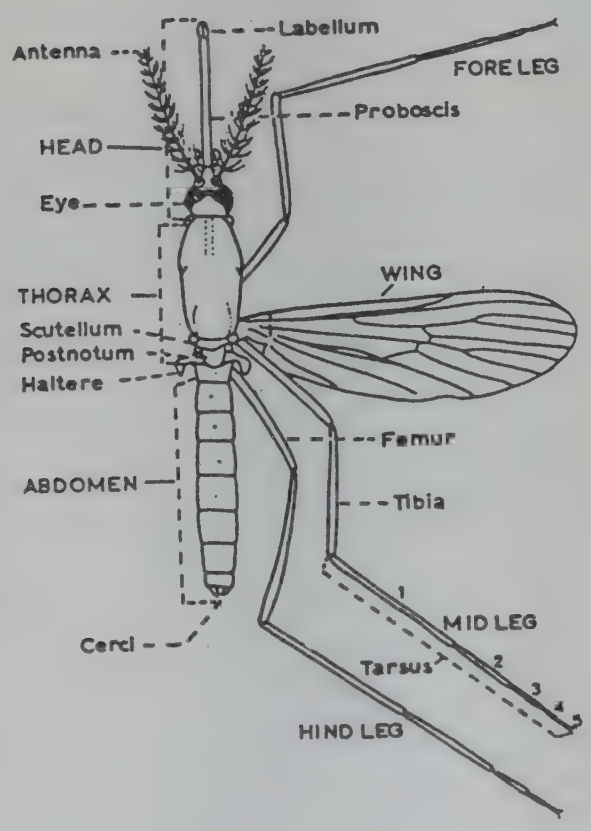


Fig 3.1

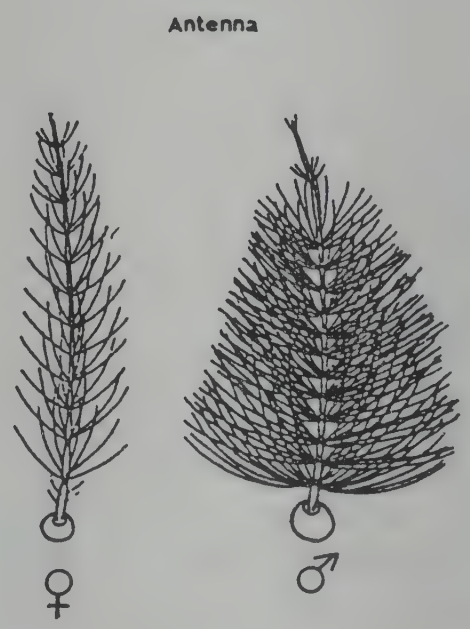


Fig 3.2

3.1. HEAD: The head bears the two large compound eyes, the long antennae, and the mouthparts, consisting of the maxillary palps and elongated proboscis. The antennae are segmented and each joint bears a whorl of hairs. In the female mosquito, these whorls of hairs are sparse and short; whereas in the male, they are dense and long and give a distinctly bushy appearance to the antennae which is visible to the naked eye (Fig 3.2). The maxillary palps also exhibit sexual variations. The proboscis is an elaborate apparatus adapted in the females of most mosquitoes for piercing and blood-sucking.

3.2. THORAX: The thorax is wedge-shaped, being broader above than below, and bears three pairs of legs on its lower surface and one pair of wings near its upper surface. It is composed of three fused segments; the prothorax, bearing the front pair of legs; the mesothorax, bearing the pair of wings and the middle pair of legs; and the metathorax, bearing the halteres or 'balancers' and the hind pair of legs.

3.3. ABDOMEN: The abdomen consists of ten segments, the last two being greatly modified for purposes of oviposition in the female and copulation in the male. The modified segments thus form the external genitalia commonly known as the terminalia or hypopygium.

4. Breeding Habitats

The breeding habitats of mosquitoes vary from large and usually permanent collections of water, such as fresh water swamps, marshes, rice fields and burrow pits to smaller collections of temporary water such as small pools, puddles, ditches, drains and gullies. A variety of natural habitats such as water filled tree holes, rock pools, water filled bamboo stumps, leaf axles, water filled split coconut husks, grinding stone and any small container or depression filled with water can serve as ideal breeding ground for freshwater breeding mosquitoes like *Anopheles* and *Aedes*. While natural habitats provide ideal breeding ground for mosquito in rural areas, man-made habitats are the major contributing factors in urban areas.

Though almost any collection of permanent or temporary water can constitute a mosquito larval habitat, they are absent from large expanses of uninterrupted water such as lakes, especially if they have large numbers of fish and other predators which are likely to feed on the mosquito larvae. They are also absent from large rivers and fast flowing streams, except that they may occur in marshy areas and isolated pools and puddles formed at the edges of flowing water.

These habitats can be classified into two major categories i.e.. polluted water and clean water habitats. The polluted water breeding habitats support breeding of *Culex quinquefasciatus* and *Armigeres* sp and these habitats are :

4.1. POLLUTED WATER BREEDING HABITATS.

4.1.1. SEPTIC TANKS : These tanks are the major means of sewage disposal in areas where underground sewage systems are lacking. Septic tanks are provided with vent pipes for the escape of obnoxious gases and outlet of excess water and these outlet pipes are often left open, mosquitoes manage to enter through these openings and prolific breeding takes place within the tank. Occasionally the excess water from these tanks instead of being diverted into soakage pits, is let into either a drain or allowed to form a puddle which in turn breed mosquitoes. (Fig 4.1)

Fig 4.1

4.1.2. STORM-WATER CANALS : These canals are designed to carry storm water, but now they are being used for disposing sewage and sullage which retards the speed of flow thereby facilitating year round *Cx. quinquefasciatus* breeding. Moreover the type of construction and indiscriminate dumping of garbage reduces the free flow of water and results in the breeding of *Cx. quinquefasciatus* which prefers to breed in stagnant and polluted water.(Fig 4.2)

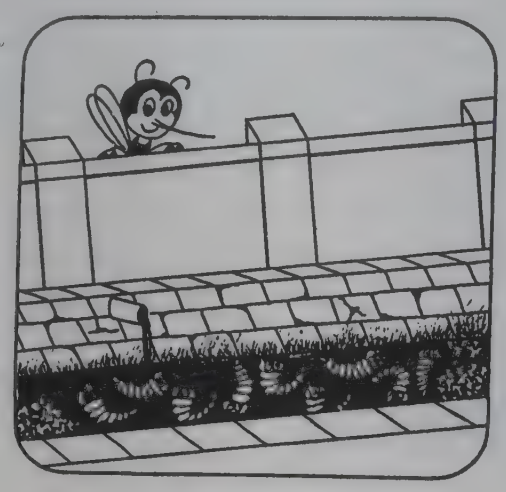


Fig 4.2

4.1.3. DRAINS : In ideal situation the drains are not suitable for breeding. However, bad engineering practices and indiscriminate dumping of garbage by the people as well as by the municipal workers result in blockage of flow in the drain. There are drains of different size and shape classified as Box drain, U-drain and unlined drain.

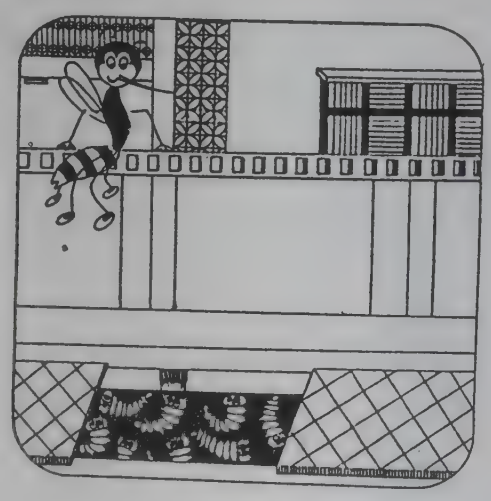


Fig 4.3

There are many U-shaped or box shaped surface drains permanently covered and most of them are blocked with garbage and silt leading to stagnation of water and breeding of the filaria vector, viz., *Cx. quinquefasciatus*. These being partly covered facilitate the entry of mosquitoes but are not accessible to cleaning or initiating any control measures (Fig 4.3).

There are cement lined U-shaped open drains. If properly constructed and cleaned regularly, stagnation does not occur. However, due to the faulty construction and dumping of garbage and other solid wastes, these drains are blocked at several points and thus they are converted into ideal breeding sites (Fig 4.4).

Fig 4.4

There are innumerable unlined drains in an urban setup and the water stagnates at several places. The flow of water may be prevented also by the growth of weeds and blocking by garbage. This stagnation of water provides an ideal breeding ground for *Cx.quinquefasciatus*. These drains can be seen on the periphery of the city and is an offshoot of unplanned urbanisation.(Fig 4.5)

Fig 4.5

4.1.4. CESSPITS: These breeding sites are highly polluted, man made and indispensable where facilities to dispose the sullage and sewage are not available. Each household digs up a pit for letting out the effluents (Fig 4.6) and as the water accumulates and stagnates, they become favourable for *Cx. quinquefasciatus* breeding.

Fig 4.6

4.1.5. CESSPOOLS : Low lying areas and vacant plots near residential area accumulate rain water and the effluents from nearby houses and finally become highly polluted to support mosquito breeding.(Fig 4.7)

Fig 4.7

4.1.6. TANKS : There are many tanks in an urban area where sullage and sewage were diverted into it from the nearby residential colonies , thus polluting them, which in turn leads to prolific breeding of *Cx.quinquefasciatus* and the growth of water hyacinth also facilitates the breeding.(Fig 4.8)

Fig 4.8

4.1.7. BREEDING ASSOCIATED WITH CATTLE SHED: It is a common site to find milch cattle in many houses and these are tethered either inside the houses or on road sides and in majority of the cases these sheds are poorly maintained and on the roadsides the flow of kutcha drains are blocked by these animals and this leads to breeding of mosquitoes. Even hoof marks of these cattle can accumulate water sufficient enough to support breeding of mosquitoes.(Fig 4.9)

Fig 4.9

4.1.8. WET CULTIVATION: In any expanding city, agriculturists grow fodder grass and vegetables by diverting the sewage and sullage water from the storm water canals which in turn stagnates at several places and provides ideal breeding grounds for *Cx. quinquefasciatus, An. subpictus* and *Cx. vishnui* group. (Fig 4.10)

Fig 4.10

4.1.9.DISUSED WELLS : Provision of protected water supply has led to the disuse of many existing wells. The dumping of garbage and letting out of effluents into these wells converts them into ideal breeding grounds for *Cx. quinquefasciatus*. (Fig 4.11).

Fig 4.11

4.1.10. COIR PITS: People in the village and semiurban areas engaged in coir making, generally soak the coconut husks in brackish water in small pits dug nearby. These coir pits form the ideal breeding ground for *Cx. sitiens* and *An. subpictus* (Fig 4.12)

Fig 4.12

4.2. CLEAN WATER BREEDING HABITATS:

Clean water breeding habitat support *Anopheles* and *Aedes* sp. Depending upon the nature of these habitats they can be classified as temporary and permanent breeding habitats.

4.2.1. Temporary breeding habitats:

4.2.1.1. PUDDLES : Leaking of public taps due to negligence and often pilferage of the taps, results in accumulation of water creating mosquitogenic conditions (Fig 4.13).

Fig 4.13

4.2.1.2. RECEPTACLES : Discarded tins, tyres, water storage mud pots (Fig 4.14), plastic containers, waste bottles, soap boxes, tar drums, flower pots and grind-

ing stones provide ideal breeding sites for mosquitoes soon after rains. Though individually each of these habitats is of minor importance but collectively their contribution could be substantial.

Fig 4.14

4.2.2 . Permanent habitats:

4.2.2.1. OVERHEAD TANKS : Majority of the overhead tanks are the ideal breeding ground for anopheline mosquitoes transmitting malaria in urban areas, when kept open.(Fig 4.15)

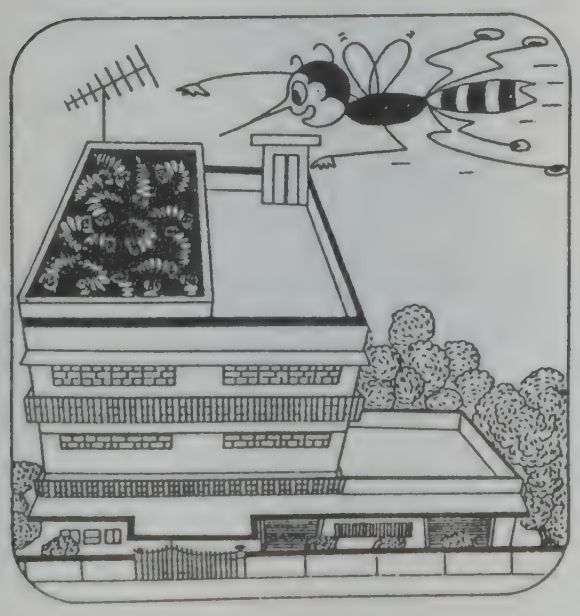


Fig 4.15

4.2.2.2. WELLS: In many areas, due to unreliability of protected water supply, people continue to use wells as a source of water (Madras, Salem etc.). These wells form the ideal breeding ground for the malaria vector, *An. stephensi* (Fig 4.16).

Fig 4.16

4.2.2.3. STREAMS: In mountainous areas, many streams crisscross the area which form the perennial breeding site for one of the efficient malaria vector, *An. fluviatilis* (Fig 4.17).

Fig 4.17

4.2.2.4. IRRIGATION CANALS: With the improvement of irrigation facilities, a network of irrigation channels has been developed to channelize the water to various fields. These irrigation canals serve as ideal breeding sites for *An. culicifacies, An. fluviatilis, An. minimus, An. maculatus* and *An. theobaldi* (Fig 4.18).

Fig 4.18

4.2.2.5. PONDS: There are large natural/man-made depressions of various sizes containing rain or subsoil water. These ponds are ideal grounds for the breeding of *An. culicifacies* and *An. fluviatilis* (Fig 4.19).

Fig 4.19

4.2.2.6. PADDY FIELDS: These fields at the initial stages of planting, form the ideal breeding grounds for *Cx. tritaeniorhynchus, An. vagus, An. subpictus* and *An. culicifaciés* (Fig 4.20).

Fig 4.20

4.2.2.7. BACKWATER: This water source known for high salinity and presence of algae generally favour the breeding of *An. sundaicus, An. vagus* and *An. subpictus* (Fig 4.21).

Fig 4.21

4.2.2.8. IRRIGATION PITS (CASUARINA AND COCONUT PITS): These are pits dug in the plantation area for irrigating the casuarina and coconut plants. These pits with fresh water are found to be ideal for the breeding of *An. culicifacies, An. subpictus* and *An. varuna* (Fig 4.22).

Fig 4.22

4.2.2.9. JUNK YARDS: Junk yards with discarded tyre dumps, old machineries, containers and other utensils favour breeding of *Aedes* sp. after getting filled with water during rainy seasons, since the eggs of this species are known to withstand desiccation (Fig 4.23).

Fig 4.23

It has been generally observed that *Cx. quinquefasciatus* breeds in highly polluted water and *An. culicifacies* and *Cx. tritaeniorhynchus* breed in paddy fields and in rain water pools. A few mosquitoes like *An. sundaicus* and *Cx. sitiens* breed almost exclusively in brackish or salt water, such as salt water marshes and mangrove swamps and are consequently restricted to coastal areas. *An. stephensi* breeds in wells, over head tanks and curing yards at the construction site and *Ae. aegypti* breeds in water storage containers.

5. Chemical Control Measures

Choice of control measure must be based on a fundamental understanding of the ecology, bionomics and behaviour of the target species and its relation to its host and environment (For guidelines, see Annexure - I). Control measures can be directed at either the immature stages or the adults, or at both stages simultaneously.

Generally larval control measures are preferred in a situation where adulticidal measures are either cost-prohibitive or nonacceptable to the community and the breeding is confined to limited area. Larval control measures are also preferred as preventive measures in areas which are prone to frequent out breaks of vector borne diseases and in situations, where, for technical

or operational reasons house spraying alone or combined with drug administration fails to interrupt disease transmission.

The control measures against adults are preferred in situations, where number of houses to be treated is less than the vast stretch of water-bodies required to be treated for anti-larval measures (mostly rural areas) or where immediate reduction of infected vector population is necessary to contain epidemics.

5.1. LARVAL CONTROL

The reduction of mosquito production should be given top priority in connection with the preventive measures, particularly in the control of permanent or consistently recurring sources. This could be achieved by appropriate naturalistic or source reduction methods. Whereas when the source is temporary, major portion of the effort must frequently be directed towards the immediate elimination of immatures through the application of chemicals.

5.1.1. Advantages and Disadvantages of chemical larviciding

Advantages:

(i) Mosquitoes developing in an aquatic habitat can be destroyed before they emerge and disperse into the areas inhabited by man
(ii) Operations can be programmed and executed within a short period.
(iii) There is a large choice of larvicides.
(iv) Operational programmes can be easily adapted and the spraymen can be trained in the techniques of larviciding.
(v) Since the breeding habitats are mostly outside the premises, acceptance by the community is better than the adulticiding.

Disadvantages:

(i) The results are of temporary in nature and a continuing programme could be cost-prohibitive in areas where there are large breeding sources.
(ii) All insecticides are known to cause health hazards due to prolonged and continuous exposure and therefore the field personnel must be trained in the safety techniques.

5.1.2. Important characteristics for a mosquito larvicide

(i) It should be highly toxic to mosquito larvae at lower dosage .
(ii) It should be rapid in its action and persistent.
(iii) Readily obtainable locally at low cost.
(iv) It should be safe to handle, transport and apply.
(v) Dffective in various kinds of water where larvae breed.
(vi) It should be non-toxic to non-target organisms.

5.1.3. Choice of insecticides suitable as larvicides

The insecticides recommended for use against the mosquito larvae and the application rates are listed in Table 5.1. Application rates vary from habitat to habitat and usually have to be increased if the water is highly polluted or contains substantial amounts of vegetation.

5.1.4. Target species and selected larvicides

5.1.4.1. *Anopheles* spp.: Paris green is recommended at the recommended application rate of 1 kg/ha. In aerial applications, 25% dust at 4.5 kg/ha and 5% granules at 17 kg/ha can be used. Temephos, an organophosphorus larvicide, formulated in 1% sand granules or 50% EC can be applied at 0.1 kg/ha in a cost-effective manner in typical anopheline breeding sites due to its large margin of safety and the low dosage required. Fuel oil can be applied at 19 - 47 l/ha with the addition of a spreading agent. The increasing cost of fuel oils,bulkyness and their limited persistence has led to a decrease in their use for anopheline control.

5.1.4.2. *Aedes* spp.: Temephos and methoprene can be applied in drinking water at 1 mg (ai) /l. Malathion, fenitrothion, pirimiphos-methyl, jodfenfos and chlorpyrifos as solutions, emulsions or granules are recommended for treatment in non-potable water at dosages indicated in Table 5.1.

5.1.4.3. *Culex* spp.: Chemicals listed in table 5.1 are recommended. The dosage should be markedly increased to provide a longer period of activity. Mostly OP larvicides are widely used. Fuel oil can be used to kill pupal stages. *Culex* spp. are generally found to

TABLE 5.1. *Insecticides suitable as larvicides in mosquito control*

Insecticide	Chemical type	Dosage g(ai)/ha	Formulation	Estimated effectiveness (weeks)	Oral LD_{50} (mg/kg) to rats
Chlorphoxim	OP	100	EC	2–7	2500
Chlorpyrifos	OP	11–16	EC, GR, WDP	3–17	135
Deltamethrin	PY	2.5–10	EC	1–3	135
Diflubenzuron	IGR	20–45	EC	1–2	4640
Fenitrothion	OP	100–1000	EC, GR	1–3	503
Fenthion	OP	22–112	EC, GR	2–11	330
Jodfenphos	OP	50–100	EC, GR	7–16	2100
Malathion	OP	224–1000	EC, GR	1–2	2100
Methoprene	IGR	100–1000	SRS	4–8	34600
Paris green	*	840–1000	Dust, Soln in oil	2	22
Permethrin	PY	5–10	EC	5–10	4000
Phoxim	OP	100	EC	1–6	1000
Pirimiphos-methyl	OP	50–500	EC	1–11	1415
Temephos	OP	56–112	EC, GR	2–4	8600

*A water soluble copper arsenite compound.

breed in confined habitats such as soakage-pits, latrines, artificial containers, etc. In the control of *Culex* spp., it has been found practicable to express dosages as mg/l rather than g/ha. For e.g., 240 ml of 0.5% fenthion or chlorpyrifos is recommended for each soakage-pit and 5 g of 10% chlorpyrifos granules are recommended for each latrine.

5.1.4.4. *Mansonia* spp.: Herbicides can be used as a last resort to destroy the aquatic weeds whose roots support the larvae because of the peculiar habits of *Mansonia* larvae. Diquat with LD_{50} (oral) value of 231 mg/kg at 0.45 l/m^3 for destroying *Eichhornia* can be used. For destroying *Salvinia*, 2,4-D with a LD_{50} (oral) value of 375 mg/kg at 400 l/ha and pentachlorophenol with a LD_{50} (oral) value of 27 mg/kg at 75-93 l/ha can be applied. For destroying *Pistia*, 2,4-D at 400 l/ha and MCPA (m-Chloro phenyl acetic acid) with a LD_{50} (oral) value of 700 mg/kg at 700 g/ha is recommended.

5.1.5. Application procedures

For applying the larvicides, generally ground equipments such as knapsack sprayers or compression sprayers (Discussed in Chapter 6, Equipments for vector control) are used.

For the control of *Anopheles* spp., the larvicides are generally applied at fortnightly intervals and the frequency of application is reduced in standing clear water and at higher dosages. Generally larviciding is not advocated when mosquito breeds in vast stretch of water-bodies. However, in exceptional situations, aerial spray with Paris green, Abate or Biocide can be attempted. It should be borne in mind that such ventures are cost-prohibitive.

In the control of *Aedes* spp., in certain cases, two to three treatments a year, carefully spaced between periods of rainfall would be sufficient. Frequent treatment is however required as a peripheral spray in and around **non-potable** containers and adjacent surfaces generally found in urban and semi-urban situations.

In the control of *Culex* spp. in slow moving water, the insecticides may be introduced by a drip technique. Drip applicator can also be used to control breeding in partially closed drains. The rate of flow of insecticide in ml/min in the drip application technique can be calculated by using the following standard formula:

$$Z = \frac{F \times G \times H \times 600}{D \times E \times J}$$

where,
Z = rate of flow of insecticide concentrate from drip applicator in ml/min
F = Width of the channel (in metres)
G = Depth of the channel (in cms)
D = Density of the insecticide concentration (mg/ml)
E = Concentration of pesticide concentrate
J = Time taken for water to flow 10 metres in seconds and
H = Concentration required in water in mg/l.

For destroying the aquatic weeds to control *Mansonia* spp., the spray should be directed vertically onto the plant in order to cover its narrow central portion. Generally two annual applications, prior to seed formation are sufficient to eliminate the plant.

5.1.6. Formulations

Generally chemical larvicides are available in EC (Emulsifiable concentrate), GR (Granular formulation), ME (Microemulsion), WDP (Wettable powder) and Oil formulations.

5.1.6.1. Emulsifiable concentrate: This is a mixture of active ingredient, solvent and an emulsifier. This is an economical form to ship high concentrations of insecticides for diluting later with water.

5.1.6.2. Granular formulation: Granules are made by impregnating or coating coarse particles with 1 - 5 % of the active ingredient. Penetration of vegetation is better obtained with this formulation.

5.1.6.3. Microemulsion: It is an improvised formulation in which the size of the particles of emulsion has been reduced by mechanical agitation to a very low level to facilitate better distribution in the treated sites.

5.1.6.4. Water dispersible powder: (WDP) This is a mixture of active ingredient, wetting agent and an inert carrier.

5.1.6.5. Solution: This is generally a mixture of the active ingredient and a solvent (aqueous or organic). Since many insecticides are insoluble in water, most solutions are prepared in kerosene or high boiling petroleum solvents. They are generally prepared on weight per volume or weight per weight.

Since most of the larvicides in use are available in Emulsifiable Concentrate (EC) formulation, the following formula will be useful in preparing working solutions for spraying.

$$X = (A/B) - 1$$

in which
X = parts of water to be added to 1 part of EC.
A = concentration of the EC.
B = concentration of the working solution.

For e.g., to prepare a working solution of temephos of 0.01% (100 mg/l) with 50% EC formulation, 4999 ml of water has to be added to 1 ml of 50% EC to get 5 litres of 100 mg/l working solution.

The same formula can be used in the preparation of working solutions with microemulsion and solution formulations.

The low-percentage-concentration granules can be applied through a granular applicator or through a tin with a few holes punched in it. The use of granules is preferred in places where water is scarce.

In the case of application of granular formulation of a larvicide, the following general formula can be used.

$$X \text{ (in gms)} = \frac{V \times Y}{10 \times C}$$

where
X = amount of granular formulation required in gms.
V = Volume of the breeding habitat in litres
Y = Concentration of the insecticide to be applied in mg/l and
C = Concentration of the granular formulation in %.

When a 0.5% GR formulation of a larvicide has to be applied at 0.1 mg/l in a breeding habitat of 400 litres of water, 8 g of Granules have to be uniformly applied. With a water dispersible powder formulation, the general formula for preparing the spray suspension has to be used.

$$X = \frac{A \times B \times D}{C}$$

in which

X = amount of water-dispersible powder required
A = percentage concentration desired
B = volume of spray suspension desired
D = 1 if X and B are expressed in kg and litres respectively and
C = percentage concentration of the water-dispersible powder

5.1.6.6. Controlled release formulations: In Urban Mosquito Control programmes, the use of larvicides and IGRs has been found to be effective for one week at the concentration of 1 mg/l. The larvicidal application needs more manpower and weekly spraying. In order to reduce the frequency of application, controlled release formulations using Cork powder,- Jiggieth (binding agent) and plaster of paris in a ratio of 1 : 2 : 4 impregnated with fenthion have been found to be effective for 1 month. These formulations are easy to apply and transport. This pellet can be applied at the rate of 1 pellet containing 4g(ai) per 100 lit of water.

5.1.7. Monomolecular Surface Films:

The monomolecular surface film producing compounds reduce surface tension and prevent the larvae and pupae from coming up to breathe and cause adults to drown as they emerge. The mode of action is purely physical which will not give rise to resistance. These are non-toxic and biodegradable which can be used in relatively low dosages of 0.2 to 1 ml/m^2.

There are three groups of products that are promising:
1. Isostearyl alcohol, or ISA 2OE, with trade name "Arosurf"
2. Sorbitan monooleate or SMO
3. Oleyl ether + cetyl stearyl ether (monoxy FCM)

5.2. ADULT CONTROL

Control measures against adults can be done in two ways by using insecticides for indoor residual treatment or space spraying. Generally residual treatment of insecticides is used to control resting insects on various types of surfaces due to 'coarse spray' and space spray is used to knock down the flying insects especially during epidemics to suppress the vector population.

5.2.1. **Residual spray:** Spraying of surface with insecticide which may persist for variable periods of time, usually months, so as to cause mortality in resting insects on contact with the treated surface.

The indoor residual treatment is preferred in situations where

(i) the basic resting behaviour of the target vector species is endophilic
(ii) the insecticide deposit should be effective for a longer period against the target species;
(iii) the insecticidal spray should not affect the resting behaviour of the vector species (irritability excito-repellency) and
(iv) the insecticidal spray should be acceptable to the local community and should conform to the sleeping habits of the inhabitants.

5.2.2. Desirable characteristics of a residual insecticide

(i) It should be effective against the vector species and the effect should last for at least three months without repelling or irritating the target species.
(ii) It should produce high mortality with 1 - 1.5 hr of contact exposure to the treated surface.
(iii) It should be less toxic to man and domestic animals.
(iv) It should be stable and should have good mixing and application qualities.
(v) It should be cost-effective.

5.2.3. Insecticides suitable for residual spray

Some of the insecticides available for indoor residual treatment against mosquito vectors are listed in Table 5.2.

5.2.4. Target species and insecticides of choice for indoor residual spray:

5.2.4.1. *Anopheles* spp.: DDT is still the insecticide of choice if the local vector mosquitoes are susceptible to this insecticide. In some areas, lindane (gamma HCH) can be used to control DDT-resistant vectors. Due to hazard to man and domestic animals, the use of dieldrin has been abandoned in most countries. Wherever, epidemiologically significant DDT-resistance is established, OP compounds, i.e., malathion, fenitrothion and pirimiphos-methyl can be used. Effective control can also be obtained with carbamate compounds like propoxur and bendiocarb and photostable pyrethroids like permethrin and deltamethrin.

TABLE 5.2. *Insecticides suitable for residual spray applications against mosquito vectors*

Insecticides	Chemical type	Dosage g(ai)/m^2	Estimated effectiveness (months)	Type of activity	Oral LD_{50} (mg/kg)
DDT	OC	1–2	6 or more	Contact	113
HCH	OC	0.2–0.5	3 or more	Contact + airborne	100
Malathion	OP	1–2	2–3	Contact + airborne	2,100
Pirimiphos-methyl	OP	1–2	2–3	Contact + airborne	1,415
Dichlorvos	OP	*	1–2	Fumigant	56
Chlorphoxim	OP	2	1–3	Contact	2,500
Fenitrothion	OP	1–2	3 or more	Contact + airborne	503
Bendiocarb	C	0 .4	2–3	Contact + airborne	55
Propoxur	C	1–2	2–3	Contact + airborne	95
Permethrin	PY	0 .5	2–3	Contact + airborne	4,000
Deltamethrin	PY	0 .05	2–3	Contact	135

5.2.4.2. *Aedes* spp.: Since 90% of this species is found to rest on non-sprayable surfaces, indoor residual treatment has been found to be of limited use.

5.2.4.3. *Culex* spp.: Since urban filariasis vector *Cx. quinquefasciatus* rest on non-sprayable surfaces such as mosquito nets, clothes, hangings and furniture and most of the other culicine species rest outdoors, the indoor residual treatment is of limited use. Larviciding is the principal method of controlling this species. Since this species has developed resistance to organochlorine and organophosphorus insecticides, carbamate insecticides like bendiocarb and photostable pyrethroids like permethrin and deltamethrin can be used.

5.2.4.4. *Mansonia* spp.: DDT is the insecticide of choice at 2 g(ai)/m^2 for 6 - 20 weeks. Only in areas where epidemiologically significant DDT-resistance has become established, OP and carbamate insecticides can be recommended.

5.2.5. Application procedures

Hand-operated equipments like hand-compression sprayers and stirrup pumps (Ref: Chapter 6, Equipments for vector control) are widely used to apply the made-up spray dilution. The frequency of retreatment depends upon the length of residual effectiveness of the insecticide at the dosage used, on the type of surface sprayed, vector bionomics, the climatic conditions and disease transmission season.

5.2.6. Formulations

Residual insecticides are generally formulated in the form of water-dispersible powders (wdp) and rarely as emulsifiable concentrates (EC). The wdp contains a surface-active agent which either enables the insecticide to become wet or disperses it instantly when water is added. The wdp is superior to emulsions and oil formulations on mud walls and other porous surfaces, because the absorption is less and more insecticide remains available on the surfaces to control resting mosquitoes. Moreover, the dry powder forms a uniform suspension free from large particles and show minimal frothing on stirring and agitation.

To prepare the spray solutions for residual applications, the spray suspension can be prepared by using the following formula:

$$X = \frac{250 \times Y}{C} \times 100$$

in which
X = weight of the formulated material required
Y = recommended application rate (g (ai)/m^2)
C = percentage concentration of WDP

The spray suspension is generally made in a compression sprayer of 10 litres capacity which can be used to cover an average surface area of 250 m^2.

For e.g., with 80% wdp formulation of bendiocarb, at the application rate of 0.4 g(ai)/m^2,

$$X = \frac{250 \times 0.4 \times 100}{80} = 125 \text{ g}$$

125 g of the formulated material should be mixed with 10 litres of water in a compression sprayer and the resulting suspension after agitation, can be used to cover a surface area of 250 m^2.

5.3. SPACE SPRAY:

The adult population can also be controlled by space spraying (atmospheric spraying of insecticide for immediate killing or knock down of flying as well as resting insects in a specified unit area) with insecticides. The prime objectives of space spraying are (i) the achievement of speedy reduction of the adult vector population and (ii) suppression of the broods otherwise destined to follow.

The space spray will generally persist in the air for an appreciable length of time and can be used to control flying insects.

The space sprays should not be recommended as a routine measure to suppress the mosquito density because,

i) The droplet size of the spray being very small may cause pollution hazards to nontarget organisms including cattle and human.
ii) The cost of the operation and maintenance of the equipment is exorbitant.
iii) The spray generally eliminates the useful predators such as spiders,lizards, Toxorhynchites etc. resulting in resurgence of vector population.
iv) The space spray may lead to misuse of fuel oil used in thermal fogging.
(v) Any temporary suppression of population by spray can be compensated by the high reproductive potential of the mosquito vector.

5.3.1. Desirable characteristics of insecticides for space spray

In selecting an insecticide for space spraying, the following factors must be considered:

(i) effectiveness against the vector species
(ii) availability;
(iii) cost;
(iv) safety and
(v) methods available for application and equipment.

5.3.2. Types of space spray

Two forms of space sprays are generally recognized: thermal fogs and ULV cold aerosols.

Thermal fogs are produced by special equipment through which the insecticide (usually dissolved in an oil with a suitably high flash point) is vaporized by being injected into a high velocity stream of hot gas. These have certain limitations. Since they cause heat-decomposition of insecticides, their use in urban areas may cause traffic hazards. They may also result in fire hazards due to leakage in gas-pipelines. An advantage of thermal fogging from the public relations point of view is the dramatic "visibility" of the operation which gives the local inhabitants a clear evidence that authorities are taking positive action against their mosquito problem.

ULV cold aerosols describe the application of minimum quantities of liquid-concentrated insecticides that will provide efficient and economical control of the target vector species.

5.3.3. Insecticides suitable for space spray

The space spraying can be interior space treatment or exterior space treatment depending upon the resting behaviour of the target species. Some of the common insecticides used for space sprays are listed in Table 5.3.

TABLE 5.3. *Insecticides suitable for space spray*

Insecticide	Chemical type	Dosage g(ai)/ha	Oral LD_{50} mg/kg (rat)
Fenthion	Organophosphorus	112	330
Chlorpyrifos	”	10–40	135
Dichlorvos	”	56–280	56
Fenitrothion	”	380–580	503
Jodfenphos	”	300–600	2,100
Naled	”	56–280	430
Pirimiphos-methyl	”	100	95
Malathion	”	112–693	2,100
Propoxur	Carbamate	53	95
Deltamethrin	Syn.Pyrethroid	0.5–1.0	135
Bioresmethrin	”	5–10	7,000
Resmethrin	”	7–16	2,000
Permethrin	”	5–10	4,000

Exterior space treatment can play an useful role to control certain exophilic vector species and interior space treatment can be used to control insects which rest on nonsprayable surfaces. Malathion, pirimiphos-methyl, dichlorvos and pyrethroids such as deltamethrin and bioresmethrin can also be applied as thermal fogs. Generally malathion is most conveniently in thermal fogging.

5.3.4. Application procedures

Exterior space treatments with cold aerosols are generally made with portable back-pack or vehicle-mounted equipment or rotary atomizers mounted on aircraft. Thermal fogging is performed by hand-carried swing-fog thermal fogger and interior cold aerosols can be made with Microsol (Ref: Chapter 6, Equipments for vector control).

Retreatments are generally required at weekly intervals during malaria transmission season. The effective downwind range for an aerosol applicator for adult mosquito control can be as much as 3.2 km when droplets of 10 - 15 um are released.

5.3.5. Formulations

ULV (Ultra low Volume) formulation of the insecticides is generally recommended for cold aerosol sprays. This is a mixture of the insecticide in a suitable solvent with a low viscosity index, i.e., the same viscosity at different temperatures, and compatible with a range of chemicals. The solvent should not have any detrimental effects on the application equipment.
For making solutions for thermal fogging, the insecticides should be dissolved in an oil of suitably high flash point.

5.4. PRECAUTIONS

Larviciding: Care must always be taken not to exceed the recommended dosage when the larvicides are applied to water which might be used by humans or domestic animals or which contain wild life.

Residual treatment: Care must be taken to protect spraymen, the public and domestic animals from unnecessary or prolonged exposure or accidental ingestion of insecticides. (Ref: Chapter 9)

Space treatment: Spraymen should be well protected to avoid exposing their skin to insecticide concentrates and should avoid inhaling the insecticide droplets. The public should be well informed about the type and period of spray to take safety precautions. (Ref: Chapter 9)

5.5. REDUCTION OF MAN - VECTOR CONTACT

5.5.1. Repellents: Repellents such as DEET and DEPA applied to skin and clothing give protection against man-biting pests for 4 - 8 hours. They are useful during visits to highly pest infested areas.

5.5.2. Insecticide impregnated nets and curtains: Mosquito nets and curtains impregnated with permethrin (0.5 g (ai)/m^2) and deltamethrin (0.025 g (ai) / m^2), the synthetic pyrethroids can reduce both man-vector contact and vector density in disease endemic areas.

5.5.3. Mosquito coils, mats and canisters: The formulations such as mosquito coils and mats can be effectively used by the communities. The active ingredient is generally a synthetic pyrethroid (d-allethrin) and the vapours released act simultaneously through their knock-down effect, repellent effect and inhibition of biting. The use of some of these formulations is limited owing to the availability of electric current and cost.

6. Spray Equipments

Insecticides will be effective only if applied precisely by the most efficient applicators. Equipment selection should be based on the type of pesticide to be applied and the size and scope of the spraying job involved. Five key factors should be considered when selecting applicator equipment.

1) Will it do the job? (Effectiveness)
2) Is it safe? (Safety)
3) Is it offensive? (Public relations aspects)
4) Is it expensive? (Cost)
5) Is it durable? (Durability)

An important consideration in the selection of spraying equipment is the size of droplets produced by the equipment during normal use. The type of the spray depends on the droplet size which is generally described by volume median diameter (VMD) expressed in micrometers (um). This is the number which divides the aerosol or spray into two equal parts by volume, one half containing droplets smaller than this diameter and the other half containing larger droplets.

Sprays may be classified according to droplet VMD as follows:

aerosols	...	50 um
mists	...	50 - 100 um
fine sprays	...	100 - 250 um
medium sprays	...	250 - 400 um
coarse sprays	. .	400 um

The equipment for the production of fine and coarse sprays generally comprise of nozzles and pumps. The knapsack sprayer, compression sprayers and stirrup pumps are most commonly used in vector control programmes.

6.1. EQUIPMENTS FOR THE APPLICATION OF LARVICIDES AND RESIDUAL INSECTICIDES

6.1.1. **Knapsack sprayer:**

This is carried on the back and a shield is provided so that it does not come into actual contact with the back. A skirt is usually fitted to the bottom of the container to prevent the direct contact with the ground.

Knapsack sprayer is a continuous type of sprayer (Fig 6.1) and the discharge rate is fairly constant. The sprayer can be used by unskilled operators with little education. Maintenance is usually simple. The operator has not only to bear the weight of the sprayer but also simultaneously to operate the pump lever with one hand and direct the spray with the other. Lighter the equipment and lesser the effort needed for operation, the better will be the spray application.

Fig 6.1

6.1.2. **Compression sprayer:**

This equipment is simple to use and versatile for vector control purposes. However as the liquid is discharged from the container, the air space increases in volume and the pressure falls. It is therefore necessary to pump air to maintain a steady working pressure.(Fig 6.2)

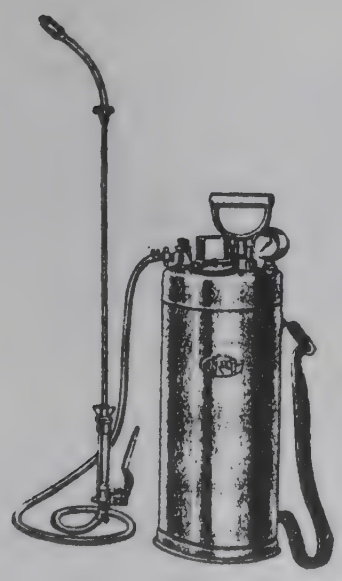


Fig 6.2

6.1.3. **Stirrup pumps:**

These sprayers are widely used in vector control programmes because they are less costly than compression sprayers. They can be used with any type of hydraulic nozzle (Fig 6.3). These sprayers consist of pump, attached discharge hose and spray lance, the pump being provided with a bracket and foot-rest or stirrup. The discharge outlet is usually placed at the top-end of the pump and should be preferably sloped downwards to prevent the delivery hose from bending or collapsing. The spray discharge is continuous because an air chamber maintains spraying pressure. This type of sprayers needs two operators, one for pumping while the other can direct the spray.

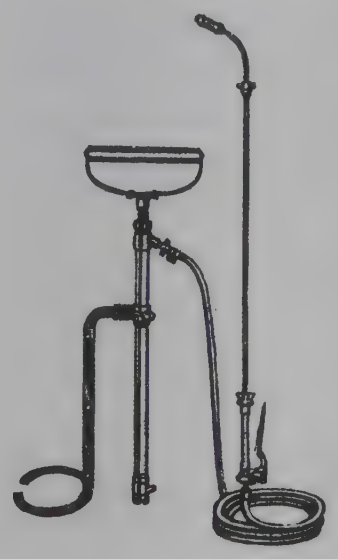


Fig 6.3

6.2. EQUIPMENTS SUITABLE FOR SPACE SPRAY:

These are either operated electrically or through internal combustion engines. Different type of equipment is necessary for thermal fogging and cold aerosol spraying.

6.2.1 **Thermal fogging equipment:**

For indoor fogging,the hand-carried thermal fogger generally called as Swing-fog can be used. In this machine, oil is injected into the exhaust gas of a pulse-jet internal combustion engine at a point where it will be completely vaporized and then immediately discharged. Devices employing this method can be hand- or shoulder-carried (Fig 6.4). But in view of the fire hazard and the possibility of misuse of diesel which is generally used, the cold aerosol sprays are preferred.

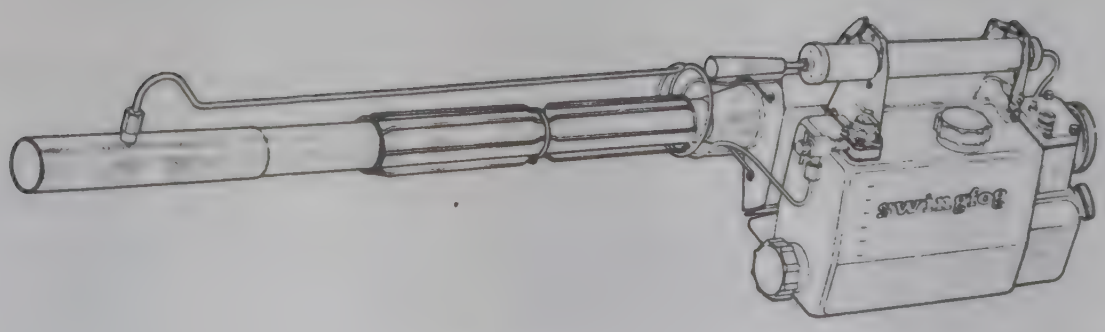


Fig 6.4

6.2.2. **Microsol:**

This is electrically operated for producing cold aerosol spray. In this applicator (Fig 6.5), vortical nozzles are employed wherein the air stream is given a rotary motion. This considerably increases its shearing action on the liquid so producing droplets in the size range of mists and aerosols.

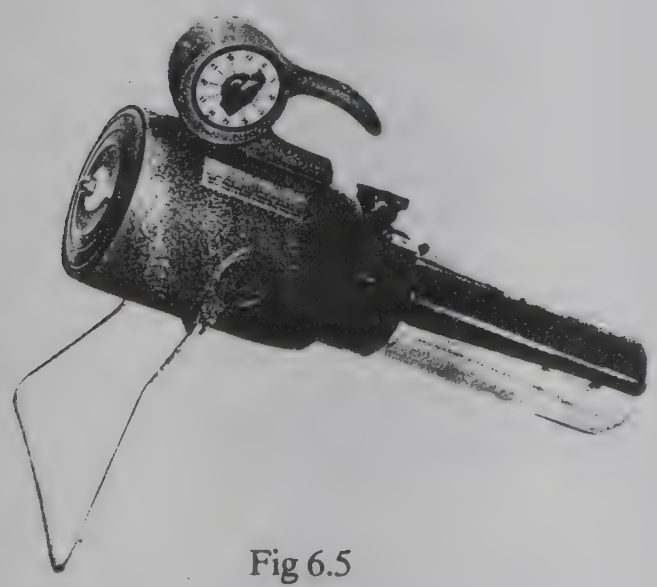


Fig 6.5

Because of the large orifices in the vortical nozzles, the problem of blockage is minimal. The popularity as a means of producing aerosols has grown to such an extent that they are replacing the thermal devices that have hitherto been used for this purpose.

6.2.3. **Mist Blower:**

Power-operated gaseous energy sprayers, or mist blowers consist of four main parts: a power source which can be either operated electrically or by internal combustion engine, a fan or blower, a pesticide container and a nozzle (Fig 6.6). The use of restrictors with small bores in the liquid feed permits the mist blower to be used for ultra-low-volume applications requiring a high air-to-liquid ratio in order to achieve the most effective atomization of the liquid.

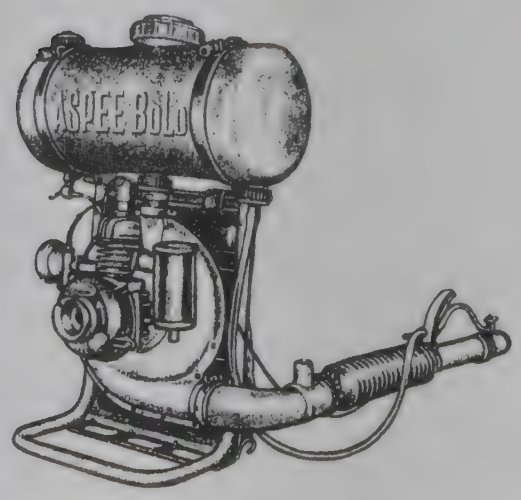


Fig 6.6

6.2.4. **LECO-ULV Generator:**

These generators overcome many of the shortcomings with thermal fogging machines. The equipment consists of a power-driven high-performance blower to supply the air, a container and a pump for the spray liquid and a directional head in which several smaller nozzles are mounted on a T-shapped head to give a wide swath discharge (Fig 6.7). These can be mounted on a trolley or vehicle. They are less noisy in operation than the thermal fogger and there is less likelihood of creating a traffic hazard.

6.3. CARE AND MAINTENANCE OF SPRAY EQUIPMENTS:

All applicator equipments require diligent care if they are to be kept operating properly. Several basic rules should be followed in the care of a sprayer.

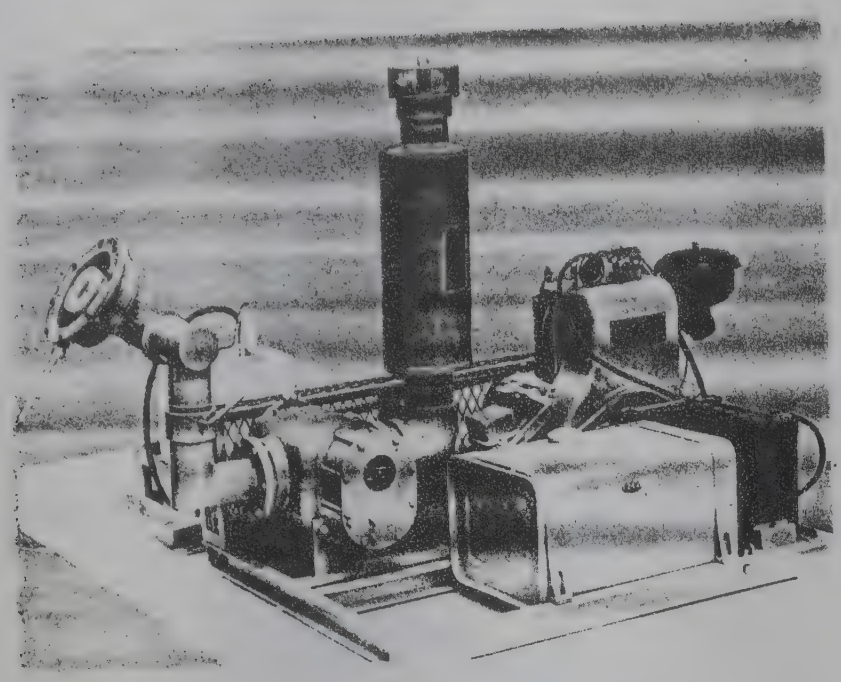


Fig 6.7

1. Handle it carefully.
2. Keep it clean.
3. Strain the formulations through proper filters.
4. Rinse it out thoroughly with water after use and pump 1 litre of water through it.
5. Every 3 months, disassemble it completely, put small metal parts into kerosene, allow to set, clean with a small bottle brush, soak nozzles, spray lance and tank with trisodium phosphate solution (washing soda), and clean with a scrubbing brush, then rinse thoroughly. Replace worn gaskets, broken parts etc. Reassemble it. Pump clean water through it.

6.4. MAINTENANCE OF POWER OPERATED EQUIPMENTS:

1. Power equipments should be covered when not in use.
2. Have regular preventive maintenance on all motors.
3. Replace damaged parts immediately.
4. Allow only experienced personnel to operate power equipment.

7. Monitoring And Evaluation

Concurrent measurement of target population is important to assess the impact of a vector control programme. This can be done either by measuring absolute density or relative density. However, absolute population measurement is not only time consuming but also not practicable in large scale control programme. Therefore,routine and continuous sampling of any stage may depict the relative fluctuation in the population thereby reflecting the effectiveness of intervention measures. Though many methods and techniques are available to measure the relative population size, no single method is perfect and has its own advantages and disadvantages.

The changes in the population size in a given area can be measured by monitoring the population of immatures,adults or both .The choice of method to evaluate the changes depends on the type of control measure and the target stages of life cycle. When control measures are directed against immatures monitoring immature population in addition to adult population is advisable. On the other hand if the control measures are directed against adults, monitoring adult population is necessary in addition to measurement of any other stage of life.

7.1 SAMPLING IMMATURES:

7.1.1. **Sampling eggs:** Monitoring number of eggs laid in oviposition sites is an ideal method to depict the relative changes in a population size. Use of simple blackjar ovitraps was found very reliable and convenient method for monitoring fluctuation in the *Aedes* population.

7.1.1.1. *Ovitraps*: Population of *Aedes aegypti* can also be detected by means of a simple device called an "ovitrap" (Fig 7.1). The ovitrap is made of flint glass with smooth tapered sides and has a capacity of about half a litre. The inside diameter at the top is about 7.5

Fig 7.1

cm and the overall height is about 13 cm. The jar is coated on the outside only with glossy black, abrasive-resistant ceramic paint and water is poured into the jar to a depth of about 2.5 cm. A "paddle" which is a strip of compressed fibre-board about (2 x 13 x 0.3 cm) with atleast one rough side is attached to the side of the jar in a vertical position by means of a clip. The jar is placed in the field. The exposed paddle is taken to the laboratory and examined under a microscope for eggs or eggs are hatched and the larvae identified. Each paddle and trap should be numbered so that positive findings can be used to locate control operations in that particular area. However, the sampling of eggs in case of *Anopheles* spp., poses a special problem as the eggs are laid in widely scattered area in damp surface and rotting debris and are not visible. The methods (Extraction method, Soaking method.) available are cumbersome and is of very little practical use. Moreover it is difficult to identify the eggs of different species at this stage. In *Culex* though it is possible to collect and count the egg rafts, the sampling procedures to be followed is not ideal. The rafts being immobile and floating tend to aggregate in few pockets. These methods of sampling egg are applicable only for studying oviposition behaviour or species composition but not useful for routine monitoring for assessing change in relative density.

7.1.2. **Sampling the larval population:**

Larval surveys reveal the sites where mosquitoes are breeding; accurate records of the surveys provide an index of the species present and indicate variations in their relative abundance.

Larval population can conveniently be sampled by dippers which are easy to use in field. A dipper can be made from a small enamel bowl which can hold 300 - 400 ml of water and a handle (1 metre length) which will be attached to the enamel bowl. The dipper should always be white inside to facilitate easy detection of larvae, particularly early instars. The number of dips that have to be taken from the water body depends upon the latter's size. One should always record the number of dips made and the number of larvae captured. The number of dips taken will depend upon the size of the breeding area, but they should be made systematically throughout the site and the total number should be a multiple of 5 or 10 for convenience in determining the number of larvae per dip.

The density of the larvae can be expressed in terms of average number of different instars per dip .The larval population can also be sampled by using floating quadrats and nets etc. A variety of techniques and equipment has been developed for sampling immatures of mosquitoes in the past. By continuing the sampling over long periods, interpretation of the results becomes more meaningful. Thus it is important to establish sampling stations in the early stages of a programme and to make regular collections.

7.2. SAMPLING ADULTS:

In general, samples of adult mosquitoes can be taken in the act of biting human volunteer "baits", or resting mosquitoes from resting sites or during their flight and swarming activity by various type of traps. The method of choice to sample the mosquitoes should ideally be simple, easy to carry out and less expensive.

7.2.1. **Sampling the resting populations:**

Adults of many species are inactive during the day and may be found resting quietly in dark, cool, humid places. A careful inspection of a daytime shelter will provide an index to the species and the population densities of the mosquitoes using it. Good collections from resting shelters are valuable indicators of the effectiveness of control measures.

7.2.1.1. *Hand Catch*: One of the simplest and easiest methods of sampling the resting mosquitoes is to collect the resting mosquitoes by an oral aspirator.An oral aspirator consists of glass or plastic tubing of about 30 - 45 cm length and 8 - 12 mm in diameter,over which a 50 cm length rubber tubing is slipped (Fig 7.2). A piece of mosquito netting or fine wire guaze is fixed

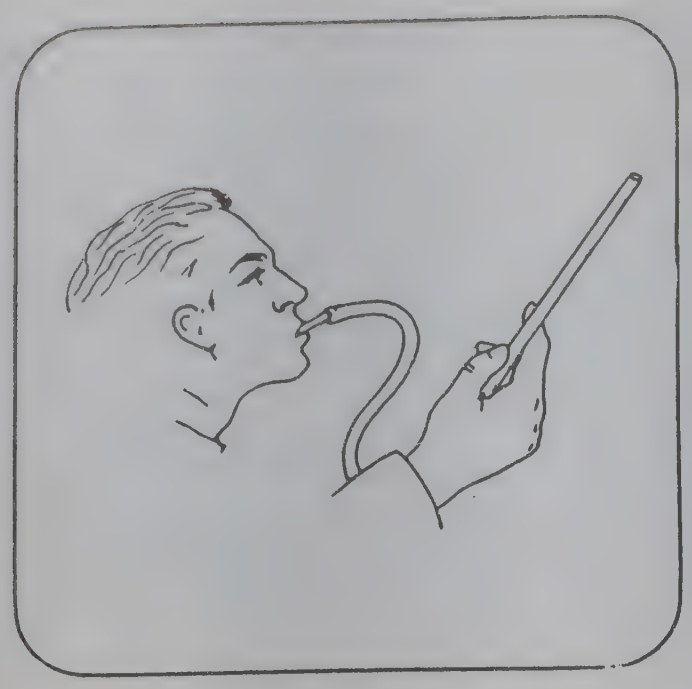


Fig 7.2

over one end of glass tube . By positioning the opening of the glass tube behind resting mosquito, they can be sucked up into the tube and later transferred to a test tube. The mosquitoes have to be collected for a fixed duration of time. The density of resting mosquitoes is generally expressed in terms of man-hour density. While calculating man-hour density only females can be taken into account as the males are epidemiologically not important. The man-hour density can be calculated as follows:

$$\frac{\text{Number of female mosquitoes collected}}{\text{Number of man-hours spent}}$$

The suspected resting places of mosquitoes should be searched thoroughly with the aid of torch lights to collect them. Mechanical aspirators, test tube or killing tube can also be used whereever possible. The killing bottle is made from a glass or plastic tube of convenient size; a large test tube about 2.5 cm in diameter and 18 cm in length is often preferred. The tube is filled to a depth of about 2.5 cm with finely chopped rubber bands, art gum, or other rapidly available form of rubber. A sufficient quantity of chloroform or ethyl acetate to saturate the rubber is then added. A disk of blotting paper is placed over the rubber, then a 1.25 cm layer of cotton-wool and finally two or three more disks of blotting paper slightly larger than the internal diameter of the tube are pressed down over the cotton-wool layer. The tube is closed with a cork stopper. Killing tubes remain effective for several weeks and can be recharged with chloroform when necessary.

7.2.1.2. *Spray Sheet collection:* Indoor resting mosquitoes can also be collected by knocking down the resting mosquitoes by insecticides like pyrethrum or any other compound with knockdown property. All the doors, windows and any other opening should be covered . Floor should be covered with few white spread sheets to collect the knocked down mosquitoes Having completed the preparatory work the room should be sprayed with pyrethrum and leave the room for 10 minutes for all the mosquitoes to be knocked down. In the cases where eves are too open and and can not be closed ,another person should spray simultaneously from outside to prevent escape of mosquitoes. After 10 minutes all the mosquitoes knocked down can be collected by entomological forceps. However this method of collection can not be used routinely because the method itself may reduce the vector population if it is done in large scale. However, in combination with hand catch collection can give a clear indication of the density as well as the efficiency of the Hand catch methods.

7.2.1.3. *Traps*: Resting mosquitoes can also be collected by providing artificial shelters which can act as a trap like black box, mud pot or pit shelters etc. Designing appropriate would take into account the resting behaviour of the mosquito and should aim at providing similar condition. However, these methods may not work in areas where ideal resting habitats are available in excess.

7.2.1.4. *Outdoor Resting Mosquitoes*: Sampling outdoor resting mosquitoes poses special problem, therefore additional tools have to be used. The actual method of collection depend upon the species to be sampled and the area. As a general guideline if the mosquitoes are resting in bushes Sweepnets, Dropnets or mechanical aspirators may be used in addition to collection with oral aspirators.

7.2.2. **Sampling the biting population:**

Collecting mosquitoes in the act of biting is a convenient method of sampling adult populations. The biting mosquitoes can be collected by using baits or baited traps. Reliable data on vector biting density can be obtained by collecting mosquitoes landing on human bait using test tubes or oral aspirators. In this method, a person rolls up his shirt sleeves and trouser exposing arms and legs and sits quitely. When the mosquito lands on the body and starts probing, the bait

himself or another person collects these mosquitoes by putting a test tube on the mosquitoes that settle on the exposed skin or by oral aspirators. The biting mosquitoes may be located by occasionally searching the bait's body with a torch light.

Biting mosquitoes have also to be collected over a period of time and is also expressed in terms of man-hour density. Biting collections become more significant as an index of vector population size when they are carried out at regular intervals by the same person, some individuals being more attractive to mosquitoes than others. For species biting in daytime, the index may be based upon the number of mosquitoes alighting upon the clothing of the volunteer bait in a given time (i.e., the landing rate).

The host seeking mosquitoes can also be collected by using light traps, carbondioxide traps ,magoon trap, bednet trap etc, efficacy of which will vary from place to place.

7.2.2.1. *Light-trap:* Light traps have been used widely to obtain data on the abundance and species composition of mosquito populations in a control area. When the mosquitoes approach the light they are sucked downwards through a screen funnel into a killing jar or mesh bag suspended below the trap. The light and fan are generally operated by electricity from the mains supply but batteries can be used in remote areas (Fig 7.3).

Experiments have shown that carbon dioxide used in conjunction with the light trap increases the number of mosquitoes and the range of species collected. About half to one kg of dry ice (solid carbon dioxide) wrapped in a double layer of newspaper or aluminium foil and suspended alongside or slightly above the trap provides sufficient carbon dioxide for one night. The technique may be particularly useful in areas where mosquitoes are scarce. The studies also shows that light traps in combination with lactic acid also increase the trap efficiency.

There is considerable variation in the attractiveness of light traps to different species of mosquito. An evaluation of light-trap collections must, therefore, be made in conjunction with other sampling methods.

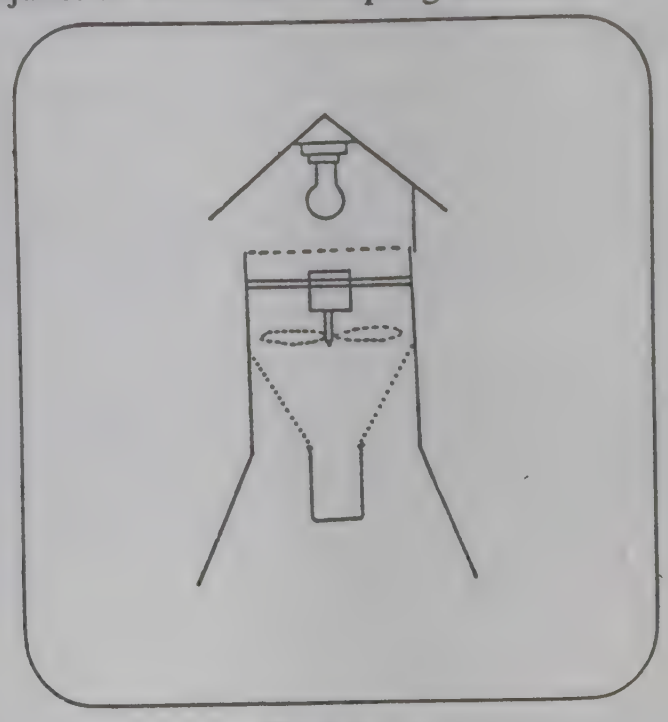


Fig 7.3

7.2.2.2. *Sampling Ovipositing Mosquitoes:* It is well known that the mosquitoes probe the water bodies prior to laying eggs. Taking advantage of this behaviour Reichter has devised a mechanical trap which sucks up the mosquitoes which come for probing oviposition sites . This trap is very successful in collecting ovipositing *Culex quinquefasciatus.*

7.2.2.3. *Sampling Swarming Mosquitoes:* Sampling mosquitoes of some species during swarming or mating dance is fairly easy and can be done with a simple sweep nets.

8. Resistance

The phenomenon of resistance has acquired global importance and has been found to hamper the success of many pest control program. Resistance occurs not only in insects against insecticides but also in bacteria, sporozoa, plants and mammals and affects a variety of toxicants including antibiotics, antimalarials, rodenticides, etc. (Fig .8.1)

8.1. VARIOUS TYPES OF RESISTANCE:

The ability of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species is defined as **resistance**. This results from continuous exposure to certain toxicants at sublethal dosage.

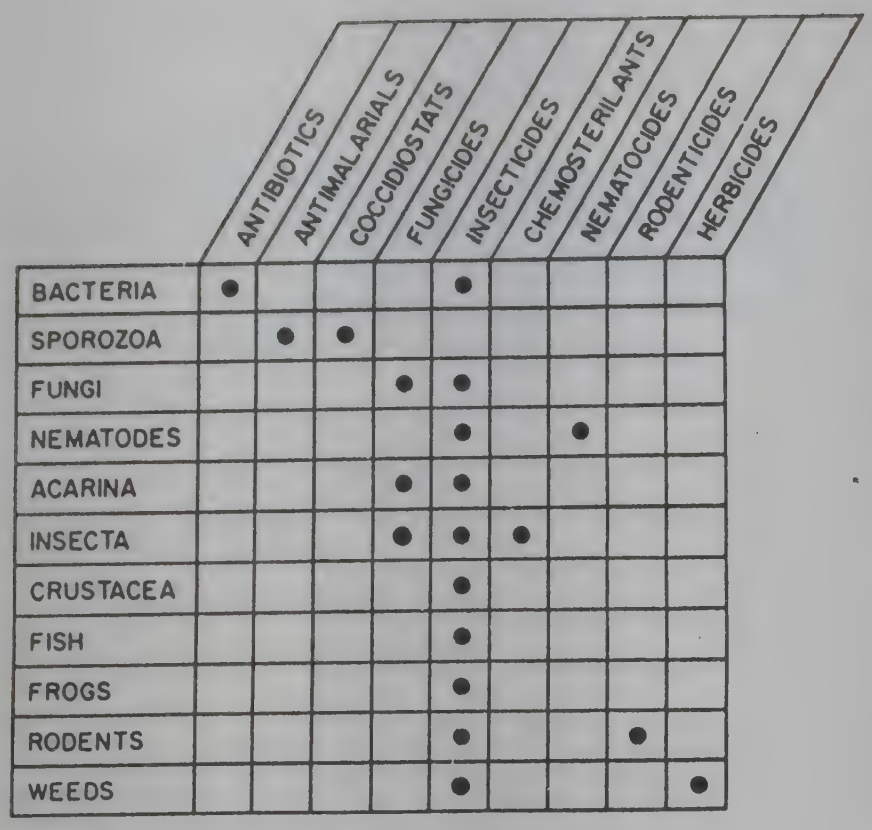


Fig .8.1

Sometimes resistance induced by exposure to one compound extends also to other compounds, and termed as **Cross resistance.**

Many insect strains develop the ability to avoid a lethal dose rather than developing a physiological indifference to the dose and such behavioural changes are termed as **Behavioural resistance**.

Similarly insects develop non-specific ability to tolerate a variety of insecticidal or environmental stresses and is usually not inherited and known as **Vigour tolerance.**

Often the insect populations are exposed to a series of unrelated chemical compounds of various classes and develop tolerance to wide variety of chemicals and such tolerance is called as **multiple resistance.**

8.2. NATURE AND EXTENT OF INSECTICIDE RESISTANCE

The development of resistance by mosquitoes to the compounds used against them as larvicides and adulticides was first observed in 1947, when the salt-marsh mosquitoes, *Aedes taeniorhynchus* and *Ae. sollicitans* began to show resistance to DDT in Florida and in the case of anophelines, resistance was first recorded in 1951 in *An. sacharovi* in Greece. Development of resistance in mosquito population has been rapid and within 40 years, 109 species of mosquito developed resistance to organochlorine; 58 species to organophosphate, 17 species to carbamate and 10 species to synthetic pyrethroids. Multiple resistance to all 4 of the above-mentioned chemical groups in the same population of a mosquito species has also been recorded in *Ae. aegypti, Culex pipiens, Cx. quinquefasciatus, Anopheles albimanus, An. culicifacies, An. psuedopunctipennis, An. sachavori* and *An. stephensi.*

8.3. MECHANISMS OF RESISTANCE

The resistance allele may be either recessive as in the case of DDT resistance or dominant as observed in some OP-resistance or co-dominant, (the resistant-susceptible hybrids being intermediate) as in Dieldrin-resistance. In a mosquito population, resistance is induced by a process of selection which increases the proportion of resistant genotypes by killing off, generation after generation, the individuals with the normal susceptible alleles.

The principal mechanism of resistant that have been identified includes:

a) enhanced metabolism of toxicant by means of enzymes like Dehydrochlorinase, mixed function oxidases, hydrolases, esterases and Glutathion-dependent transferases.

b) reduced sensitivity of the target sites by

   (i) Nerve insensitivity
   (ii) Acetyl cholinesterase insensitivity

c) reduced penetration of insecticide to active site.

The resistance against DDT is mainly due to an increase in the enzyme DDT-dehydrochlorinase, (Clark and Shamaan, 1984) which detoxifies DDT to DDE. Another mechanism of DDT-resistance is nerve insensitivity, due to a knockdown-resistance (kdr) gene which also confers pyrethroid resistance. Kdr gene alters the nerve ultrastructure by reducing the number of receptors for DDT and pyrethroids.

Resistance to cyclodiene group of compounds (e.g. Dieldrin) is associated with a deficiency in those receptors in nerves which are blocked by cyclodienes as well as by picrotoxinin. A decrease in the number of these receptors results in the dieldrin-R types having less binding affinity for the cyclodiene compounds or gamma-HCH so that the target sites have less chance of being blocked by them (Kadous *et.al.,* 1983).

Resistance to most of the OP compounds is due to phosphatase-type hydrolysis of toxic organophosphorus compound by esterase isozyme. Whereas in the case of malathion, hydrolysis is by carboxylesterase. In the case of malathion and fenitrothion, certain degree of resistance is also due to insensitivity of Acetyl Cholinesterase. It was observed that insensitive AChE was 5 times more slowly inhibited by malaoxon or fenitroxon than a normal strain.

Carbamate-resistance is partly due to the AChE being insensitive to carbamates, (in *An. albimanus* to propoxur) and due to increased detoxication by oxidative enzymes (in *Cx. quinquefasciatus* topropoxur).

8.4. TEST METHODS TO DETER MINE SUSCEPTIBILITY OR RESISTANCE

The function of susceptibility-resistance tests is mainly to detect the decay in susceptibility level and the emergence of resistance.

8.4.1. Larval tests:

For assessing the development of resistant in the test populations against the larvicides used to control, the standard procedure for determining the susceptibility to the larvicides has to be followed:

(a) Test concentration should be arrived by adding 1 ml of the standard in ethanol to 249 ml of water. Concentration of the standard solution needed for particular concentration can be arrived at by the following formula:

Concentration of std stock soln x 1 ml of std = Concentration of test soln x 250 ml of test soln.

For e.g., to prepare a test solution (250 ml) of 1 mg/l, one needs a 250 mg/l of standard solution from which 1 ml has to be pipetted out and diluted with 249 ml of water.

(b) Atleast four replicates for each concentration should be set up with a control with 1 ml of ethanol with 249 ml of water.

(c) Add 1 ml from the standard solution that would give the desired test concentration to the beaker. The beakers should be selected in such a way that the depth of water remains between 2.5 and 7.5 cm.

(d) Lots of 20 - 25 IV-instar larvae should be taken in 25 ml of water in 50 ml beakers. They must be left to rest for 15 - 30 min. in these beakers for acclimatizing to the experimental conditions. At this stage, un healthy/parasitized or damaged larvae should be rejected. The selected test larvae should be transferred to the test beakers containing 249 ml of water with the help of a flat strainer.

(e) Observation should be made on mortality after 24 hours. Moribund larvae (those incapable of rising to the surface) are considered as dead larvae and should be added to the dead larvae for calculating the percentage mortality.

(f) Discard the larvae that have pupated during the test. If more than 10% of the control larvae pupate in the course of the experiment, the test should be repeated. Tests with a control mortality of 20% or more should also be repeated.

(g) If the control mortality lies between 5 and 20%, the corrected mortality percentage from the observed mortality percentage can be obtained by using Abbotts' formula

$$\text{Corrected mortality}(\%) = \frac{\text{Observed mort. (\%)} - \text{Control (\%)}}{100 - \text{Control (\%)}} \times 100$$

After getting the baseline figures, routine surveillance and monitoring for resistance can be made by tests at a single concentration which is just sufficient to ensure complete kill as indicated by the regression line for the normal population of base-line susceptibility.

To serve as a guide, the WHO (1980) has proposed, on the basis of experience of many years, the following tentative diagnostic dosages (in mg/l):

	Mala-thion	Teme-phos	Fenth-ion	Fenitro-thion	Propoxur
Aedes aegypti	1.0	0.02	0.05	0.06	0.01
Culex pipiens complex	1.0	0.02	0.05	0.125	0.01
Anopheles spp.	3.125	0.25	0.05	0.125	0.025

Survival of any larvae from exposure to the diagnostic dosage would indicate the possibility of resistance among the population tested and the necessity of performing the detailed multiple- concentration test to validate the results.

Test for Insect growth regulators (IGRs or juvenoids): The test method is similar to that used for larvicides except that larvae are exposed to the test concentrations continuously till they emerge into adults. During the testing, the mortality in the different moulting stages, viz., larval-pupal intermediates and pupal-adult intermediates has to be monitored. Any abnormality in the different moulting stages should also be observed. The percentage of emergence inhibition (EI) for the particular concentration should be found out. The standard solutions of methoprene and diflubenzuron for the test concentrations, 0.002, 0.004, 0.001 and 0.25 mg/l have to be tested against the fourth instar larvae.

8.4.2. **Adult tests:** Resistance in adults can be monitored by methods prescribed by WHO using the standard WHO test kit.

8.4.2.1. *Composition of the test kit:*

(a) 20 plastic tubes, 125 mm in length and 44 mm in diameter. Eight of these (with a red dot) are used for exposing the mosquitoes to the insecticide; 8 (with a green dot) are used as holding tubes; 2 (with a green dot) are used as holding tubes for pretest sorting and post-exposure observation (control). Each tube is fitted at one end with a 16 mesh screen. In order to identify the exposure time used with them, the red exposure tubes should be numbered 1 to 8, the green control exposure tubes 9 and 10 and the holding tubes 1a to 10a.

(b) 10 slide units, each with a screw-cap on either side and provided with a 20mm filling hole.

(c) Insecticide impregnated papers (12 x 15 cm) of different concentrations can be prepared in olive oil base using Whatman No. 1 filter paper.

(d) Sheets of plain paper (12 x 15 cm) for lining the holding tubes.

(e) 20 spring wire clips to hold the papers in position against the walls of the tubes. The 12 silver clips should be used only for the holding tubes and the control exposure tubes; the 8 copper clips should be used only for the insecticide exposure tubes.

(f) 2 glas aspirator tubes, 12 mm in internal diameter, together with 60 cm of tubing.

(g) 1 roll of self-adhesive plastic tape.

8.4.2.2. *General conditions for testing*:

Paper: Insecticide impregnated paper may be used for upto 20 times and/or upto 3 weeks provided all precautions are taken against evaporation of oil. The paper could be left in the tubes, with the open end well wrapped and placed in a cool place. Paper should not be stored in a refrigerator as too low a temperature may cause crystallization in higher concentrations.

Mosquitoes: Fully fed female mosquitoes should be used.

Experiment: The experiment should be done in rooms free from insecticidal contamination and extremes of temperature, humidity, illumination and wind.

Procedure:

(a) Insecticide impregnated papers of diagnostic concentrations should be inserted into each exposing tube and fastened along the wall of the cylindrical tube with a spring wire clips. The clips used for exposure tubes and holding tubes should have a differential marking. Ordinary white papers can be inserted into the holding tubes and fastened along the wall by a wire clip. Attach the slides to the tubes.

(b) Introduce suitably selected female mosquitoes (10 to 20) into the holding tube through the filling hole in each tube.

(c) Allow the mosquitoes in the holding tubes to stand for about 30 min to attain normal behaviour.

(d) Connect the exposure tube with the insecticide impregnated paper to the holding tube.

(e) Transfer the mosquitoes to the exposure tubes through the bigger opening in the slide and close the passage between the holding and exposure tubes by pulling the slide.

(f) The exposure tubes should be kept upright for the required exposure period.

(g) After the specific period of exposure, transfer the mosquitoes back to the holding tubes and keep the holding tubes with glucose padding for 24 hr. The temperature and humidity should be maintained optimally, i.e., 28 $\pm$ 1 °C and 70 $\pm$ 10%.

(h) Mortality counts should be made after 24 hr period. Utmost caution should be taken while handling the mosquitoes.

(i) Atleast four replicates for each diagnostic concentration should be set up.

The tentative diagnostic concentration and exposure time for different insecticides proposed by the World Health Organization (1980) are as follows:

	DDT	Dieldrin	Malathion	Fenitrothion	Propoxur
Anopheles spp.	4 % 1 hr	0.4 % 1 hr	5 % 1 hr	1 % 2 hr	0.1 % 1 hr
Culex quinquefasciatus	4 % 4 hr	4 % 1 hr	5 % 1 hr	1 % 2 hr	0.1 % 2 hr

Results of exposure to diagnostic dosage should be interpreted as per the guidelines provided below:

98 % mortality = susceptible,
80 - 98 % mortality = verification required,
80% mortality = resistant individuals present

For verification, females of the F_1 progeny from the survivors should be tested. If they show less mortality than the parent generation (P), it may be concluded that resistance is present.

Susceptibility test results are of the greatest value in anticipating or confirming resistance, but it is the combination of field observations of control failure with the test results which add up to what can be called a true case for resistance, i.e., to the recommended application rate of the insecticide.

8.5. TESTS FOR RESISTANCE MECHANISMS AND CROSS-RESISTANCE

A direct approach to elucidate the resistance mechanisms involved in a given sample of test species is by studying the electrophoresis zymograms with a kit containing the chromogenic means of testing for carboxylesterase, MFOs and glutathione S- transferase and of differentiating between esterases. A less- sensitive AChE target enzyme is another resistance mechanism which may be found in any species of anopheline or culicine; indeed, the three genotypes for the Ace gene which determines this resistance mechanism may be distinguished in *Cx. q. pipiens* by a single-mosquito test (Raymond *et al.*, 1985). Syner-

gists may also be used as diagnostic tools, piperonyl butoxide to reveal MFOs, DEF (S.S.S-tributyl phosphorotrithioate) to reveal esterases and F-DMC to reveal glutathione S-transferase.

8.6. COUNTERMEASURES FOR RESISTANCE

Ever since resistance was first suspected in field populations, the possible fate of a given insecticide against a given species has been probed. From studies, it was found that resistance could be reverted on relaxation of pressure in most cases. Since resistance is mainly due to selection pressure, various measures can delay the precipitation of resistance or reverse it.

8.6.1. **Resistance management:** General principles to minimize the resistance problem (Metcalf, 1983) include:

1. avoiding insecticides that select for resistance to other insecticides also.

2. as a general rule, avoiding mixtures of insecticides, thus inducing more than one type of resistance at the same time;

3. avoiding the use of the same insecticide treatment against adults as that used against larvae.

Detoxication enzyme	resistance in	synergist
Esterases:		
Carboxylesterase	Malathion	IBP & TPP
Phosphatase	OP-resistance	DEF
Carboxylamidase	OP (Dimethoate)	DEF
Microsomal mono-oxyg	OP & carbamate	PBO
Dehydrochlorinase	DDT	FDMC

IBP = S-Benzyl-O,O-diisopropylo phosphorothioate
DEF = S,S,S-Tributyl phosphorotrithioate
TPP = Triphenyl phosphate
PBO = Piperonyl butoxide
FDMC = Chlrofenethol

8.6.2 **Change of insecticide:**

The ultimate countermeasure for a case of resistance is to switch to another insecticide, if not to an entirely different method of control. The policy considered most acceptable has been to continue with one insecticide until the susceptibility test results indicate that resistance, in terms of a control failure, is imminent (Metcalf, 1983). The weapon in reserve may be in an entirely new chemical group (e.g. pyrethroids) but the most common situation is the necessity to switch from one OP to another.

The criteria for selecting a compound is that the compound should give the least cross-resistance and reserving those which induce a cross-resistance as a last resort. For residual insecticides in anopheline control against malaria, it is best to start with malathion and hold fenitrothion or pirimiphos-methyl in reserve because the mechanism for the development of resistance against malathion is selective and would not induce cross resistance to other OP compounds.

8.6.3. **Synergists**: The following well-known synergists can be used with the insecticides where development of resistance in the target population has been due to the particular detoxication enzyme. For example, resmethrin synergized with piperonyl butoxide (1:3) was effective for quick adult kill of malathion-resistant *Ae. sollicitans* in New Jersey (Sutherland *et al.*, 1983).

8.6.4. **Mixtures**: Combinations of two or more different insecticides are seldom considered, since they tend to produce more than one resistance simul taneously. A notable exception was found in a susceptible compounded California strain of *Cx. quinquefasciatus*, in which selection with a permethrin-temephos larvicide mixture failed to induce any resistance;

moreover, the temephos-resistance induced by temephos selection was abolished by subsequent permethrin selection and vice versa (Georghiou *et al.*, 1980).

8.6.5. **Mosaic or Grid pattern**: An important variation in the concept of mixtures for vector control is the application of unrelated insecticides in different sectors of a 'mosaic' or grid pattern. The objective of this strategy is to avoid selection of the population for the same resistance mechanism in all regions of the treated area so that migrants that have not been killed in their sector of origin will be killed upon exposure to the insecticide used in the adjoining sector. (Fig. 8.2)

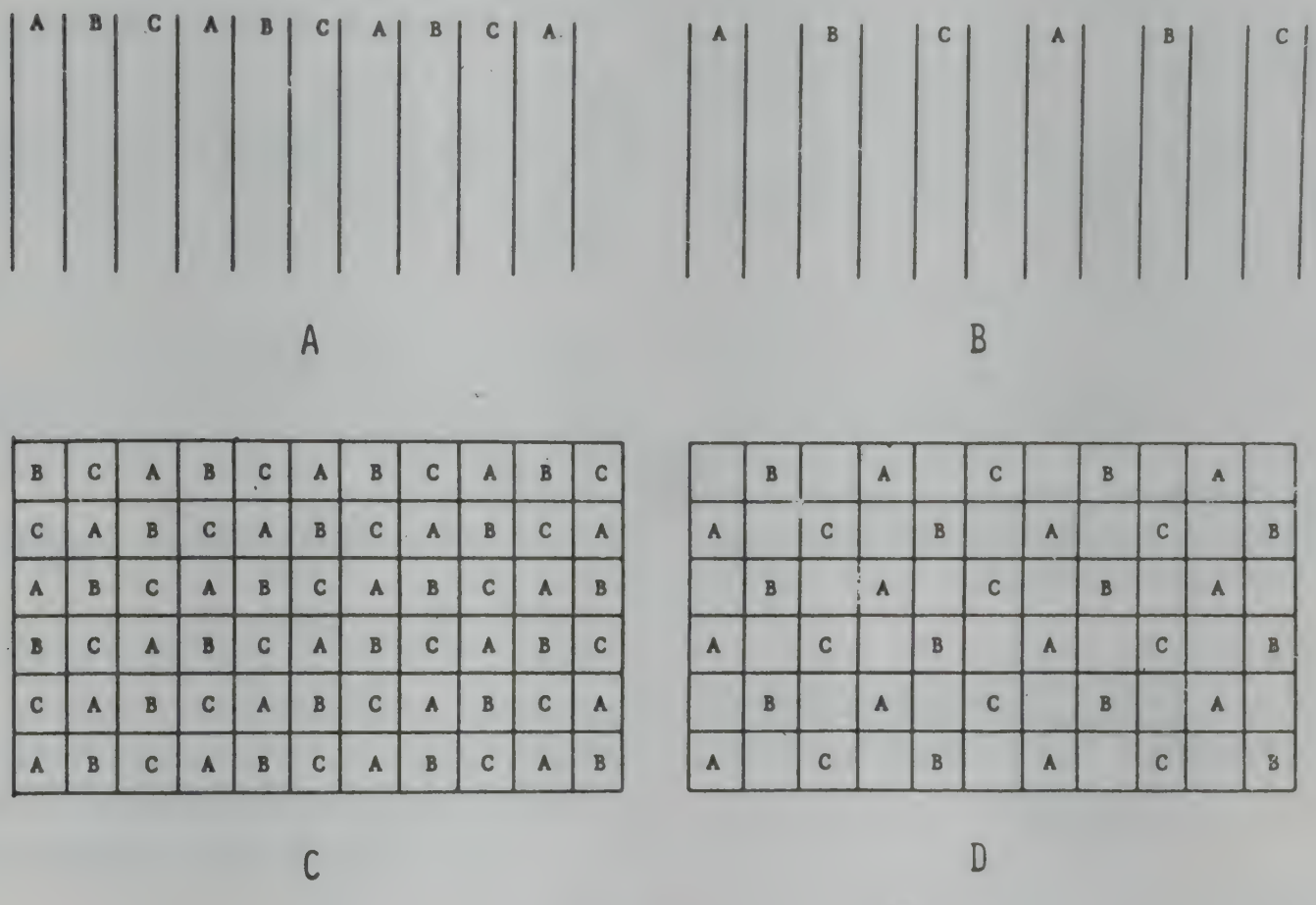


Fig. 8.2

8.6.6. **Alternation or rotations**: To delay the onset of insecticide resistances in *Cx. quinquefasciatus*, some success has been obtained with an arsenal of 5 entirely different insecticides, namely propoxur, temephos, permethrin, diflubenzuron and Bti, when the changes were made after 5-9 generations (Georghiou *et al.*, 1983). Long-term sequential selection, where the change of insecticide is arbitrarily made several generations after its introduction, has an advantage over the practice of waiting until resistance develops before making a switch.

Bearing in mind, the genetic, biological and operational influences on resistance development, the concept of resistance management has been developed by Georghiou (1980). Three types of resistance management strategies have been proposed by Georghiou (1983).

Chemical strategies of resistance management

Type A Management by moderation

Low dosages, sparing a proportion of susceptible genotypes
Less frequent applications
Chemicals of short environmental persistence
Avoidance of slow-release formulations
Selection directed mainly against adults
Localized rather than area-wide applications
Certain generations or population segments left untreated
Preservation of "refugia"
Higher pest population threshold for insecticide application

Type B Management by saturation:

Rendering R gene "functionally" recessive by higher dosages on target
Suppression of detoxication mechanisms by synergists

Type C Management by multiple attack:

Mixtures of chemicals
Alternation or rotation of chemicals

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9. Hazards of Pesticide Usage & Safety Precautions

The vector control involving pesticides though effective and beneficial to the individual and community at large, has certain hazards as well. Almost all chemical pesticide may evoke certain undesirable effects. Such effects may be **acute**, which normally occurs shortly after contact with a **single dose** of poison or **chronic**, which occurs after an exposure to **repeated small and non-lethal doses** of pesticides. The risks to man, domestic animals, and wildlife include possible poisoning or even death. Another risk is the contamination of air, water, food or soil.

However, by proper selection of compounds and by their proper use, all these risks can be reduced and prevented to a greater extent.

9.1.ROUTES OF ENTRY OF INSECTICIDES

9.1.1. **By Mouth:**

Dusts and sprays entering mouth during applications.
Drinking pesticides from unlabelled or contaminated containers.
Using the mouth to start siphoning of liquid con centrates.

Eating contaminated food.
Transfer of chemical to mouth from contaminated cuffs or hands.
Drinking from contaminated beverage container.

9.1.2. **Through the skin**:

Accidental spills on clothing or skin.
Dusts and sprays settling on skin during plication.
Splash or spray in eyes and on skin during pouring and mixing.
Spraying in wind.
Contact with treated surfaces.
Children playing in discarded containers.
Maintenance - repair work on contaminated equipment.

9.1.3. **By breathing**:

Dust, mists or fumes.
Smoking during application or contaminated smoking supplies

9.1.4. **Inoculation**:

Through cuts, abrasions and rashes of the skin.

9.2. HAZARD IN RELATION TO HANDLING PESTICIDES

Hazard is generally defined as the the risk of poisoning arising in handling the pesticides in practice. Thus the most toxic insecticides may be handled with little hazard if sensible and disciplined adherence to good practice becomes routine. Hazard is a function of two other variables besides toxicity, contamination and time and is expressed by an equation.

Hazard = Toxicity x Contamination x Time

where
Hazard is the risk of poisoning
Toxicity is the ability to cause damage
Contamination is the prerequisite for entering body
Time is the duration of contact with insecticide.

Hence, if any one of the three variables on the right side is zero, the hazard will also become zero. Hazard due to the handling of the insecticide can be minimized by reducing the variables on the right side of the equation.

9.2.1. **Toxicity**

Toxicity is an innate capacity of a chemical to cause damage and may be expressed in terms of LD_{50} (mg/kg) as (i) **oral toxicity,** the reaction following ingestion of the material by mouth; and (ii) **dermal toxicity,** the reaction caused by absorption of the chemical through the skin or other tissues.

The data on acute oral toxicity have been used to divide insecticides into four groups (Table 9.1). These groupings have considerable practical value because manufacturers must label each package of insecticide with key signal words such as "Danger", "Poison" and list antidotes where required or other necessary precautions.

LD_{50} toxicity values are useful in making comparisons of the inherent toxicity of different compounds. The oral LD_{50} and the dermal LD_{50} values of a given insecticide need not be the same.

The oral and dermal toxicities of selected public health insecticides for indoor residual treatment are given in Table 9.2.

Similarly the oral and dermal toxicities of selected larvicides are given in Table 9.3.

9.2.1.1. *Methods of reducing risk*:

The selection of a proper pesticide is a prime requisite for any effective vector control programme. In this regard, four main factors, cost, efficacy, safety and acceptability should be considered. The user is therefore intended to select the insecticide that is most effective, economical and acceptable to the community. The relationship between mammalian and pest toxicity usually offers a better indication of risk, i.e., the higher the ratio between the mammalian and pest LD_{50}s, the lower the actual risk to man.

The hazard in handling the pesticide in relation to toxicity can be reduced by:

1. Selecting material of favourable mammalian versus insect toxicity ratio,

2. Using insecticide with lower dermal toxicity

3. Using the least toxic formulation and

4. Using the lowest feasible concentration.

TABLE 9.1. *Classification of insecticides according to acute oral toxicity* (LD_{50} (mg/kg))

Classification	Acute Oral LD_{50} (mg/kg)	Signal Word and Antidote statements
Extremely toxic	0–5	"Danger", "Poison" Skull cross-bones Antidote statement "Call physician immediately" "Keep out of reach of children"
Highly toxic	5–50	"Warning" No Antidote Statement "Keep out of reach of children".
Moderately toxic	50–500	"Caution" No Antidote statement "Keep out of reach of children".
Slightly toxic	>500	No warning, caution or statement. Unqualified claims of safety are not acceptable. "Keep out of reach of children".

TABLE 9.2. *Oral and dermal toxicities of residual insecticides for public health*

Insecticides	Acute Oral toxicity to female rats LD_{50} (mg/kg)	Acute Dermal toxicity to female rats LD_{50} (mg/kg)	Recommended rate of application for residual g(ai)/m^2
Highly toxic:			
Dieldrin	46	60	0.5
Moderately toxic:			
DDT	118	2510	1–2
Lindane (gamma HCH)	100	900	0.02–0.5
Dichlorvos*	56	75	0.02–0.03
Fenitrothion	100	—	1–2
Propoxur	95	>2400	1–2
Bendiocarb	55	—	0.4
Deltamethrin	135	—	0.05
Slightly toxic or Low-order Toxicity insecticides:			
Malathion	2100	>4444	1–2
Pirimiphos-methyl	1415	2000	1–2
Chlorphoxim	2500	—	2
Permethrin	4000	—	0.5

TABLE 9.3. *Oral and dermal toxicities of larvicides for public health*

Insecticides	Acute Oral toxicity to female rats LD_{50} (mg/kg)	Acute Dermal toxicity to female rats LD_{50} (mg/kg)	Recommended rate of application g(ai)/ha
Highly toxic:			
Paris Green	22	22	840–1000
Moderately toxic:			
Chlorpyrifos	135	2000	11–16
Fenthion	330	330–500	22–112
Deltamethrin	135	—	2·5–10
Slightly toxic or Low-order toxicity:			
Malathion	2100	>4444	224–1000
Pirimiphos-methyl	1415	2000	50–500
Permethrin	4000	—	5–10
Temephos	8600	>4000	56–112
Larvicidal oil	negligible	—	19–47 lit.

9.2.2. **Assessment of Exposure (Contamination)**

Since the hazard due to a pesticide depends on the extent and period of exposure of an operator, assessment of exposure has to be determined for the realization of hazard in the handling of a particular pesticide. This is essential because the hazard can be considerably reduced even in handling a toxic pesticide, if it is applied by an experienced operator, properly equipped and protected.

Many methods have been developed to measure exposure to various insecticides but very few have been found practical for routine work. Direct monitoring methods include the determination of the active material with which the sprayman comes into contact and which is likely to be absorbed. Indirect methods are based on the measurement of effects produced by the absorbed insecticide.

9.2.2.1. *Direct method for determining the dermal exposure*:

With disposal overall/gauntlets: To assess dermal exposure, a worker is required to wear a new disposable overall and gauntlets for a minimum period of 1 hour during any one day's spraying. If significant exposure of the head is likely, a head pad or a disposable hat should be worn. Care should be taken to ensure that the overall or gauntlets do not become saturated with pesticide spray. If this occurs, a fresh one should be worn. The exact duration of exposure and amount of pesticide used must be accurately recorded. At the end of each assessment period, the overalls and gauntlets should be collected separately in plastic bags and analyzed.

9.2.2.2. *With exposure pads:* Exposure pads consists of pieces of whatman No. 1 filter papers (10 cm x 10 cm) that are backed with glassine paper or aluminum foil. When monitoring the degree of exposure to oily formulations, aluminum foils can be used. Seven such pads are fixed to the clothing of sprayman before spraying operation. Pad 1 (on the hat), Pad 2 (on back,

between the shoulder blades), Pad 3 (on chest), Pad 4 (left forearm), Pad 5 (left thigh), Pad 6 (left shin) and Pad 7 (to the skin on the abdomen under the clothing). After the spray operation for a known period, the concentration of the insecticide absorbed in the different exposure pads should be analyzed by HPLC or GC. Total dermal exposure can be calculated by the following formula:

Total dermal exposure (ug) =

Pad 1 x 837 sq.cm. + Pad 2 x 100 Sq.cm. + Pad 3 x 149 sq.cm. + Pad 4 x 1209 sq.cm. + Pad 5 x zero + Pad 6 x 800 sq.cm. + Pad 7 x 15207 sq.cm.

9.2.2.3. *Indirect method for monitoring the levels of* exposure:

1. By analyzing the levels of compounds and metabolites in blood and urine after the standardization of analytical techniques of the compound and its metabolites by GC (Gas chromatographic) and HPLC (High Performance liquid chromatographic) techniques.

2. Since OP and carbamate insecticides are cholinesterase inhibitors, it is possible to determine the degree of absorption of these insecticides by measuring the level of cholinesterase activity. This can be done by two different methods.

a) Edson's method: This method makes use of the principle in the measurement of the change in pH in a reaction mixture. The blood cholinesterase hydrolyses acetylcholine liberating acetic acid. The rate of this reaction is measured by the range of colour change in the indicator, viz., Bromothymol blue present in the solution. The change in colour produced by change in pH is matched against the colour on a comparator disc. The final result indicates the proportional reduction of activity from the pre-exposure activity in percentage. If the inhibition is 2%, the operator should be warned about the precautionary measures and if the inhibition is 50%, the sprayman should be withdrawn from the spray operation and any other exposure to insecticide.

b) Ellman's method: This method works with a principle in measuring the enzyme activity by determining the rate of thiocholine formation due to enzyme hydrolysis of acetylcholine used as substrate. Thiocholine reacts with a reagent, dithio-bis nitro benzoic acid forming a yellow complex, the intensity of which is measured spectrophotometrically. The more yellow complex formed in a unit time indicates the higher enzyme activity.

9.2.2.4. *Protection to spraymen*

The various items of protective clothing that may have to be used are described below:

(i) **Hats**: These should be of impervious material with a broad brim to protect the face and neck.

(ii) **Veils**: A plastic mesh net will afford adequate protection of the face from the larger spray droplets and permit adequate visibility.

(iii) **Overalls**: These should be of light durable cotton fabric. They must be washed regularly, the frequency depending on the pesticide being used. Washing soap, detergent should be adequate for OP and carbamate compounds. A rinse in kerosene for the OC compounds.

(iv) **Aprons**: Rubber or polyvinylchloride aprons should be used to protect from spills of liquid concentrates.

(v) **Rubber boots**: These will complete the protection afforded by the aprons.

(vi) **Gloves**: Polyvinylchloride or rubber gloves should be used when handling concentrates but unsuitable for continuous wear. Cotton gloves offer some protection for hands but should be regularly washed. Impervious gloves must be washed regularly inside and out.

(vii) **Face masks**: Masks of gauze or similar material are capable of filtering the particles from a WDP spray and may be worn to reduce inhalation of the spray and dermal exposure of the face. They must be washed regularly.

(viii) **Respirators**: These are designed to protect operators fogging with very toxic pesticide formulations. Respirators are generally not required for normal vector control operations.

9.2.2.5. *Personal hygiene*

(i) Spraymen should be provided with atleast two uniforms to allow for a change when required.

(ii) Washing facilities with sufficient water and soap should be made available in the field at appropriate location.

(iii) All working clothes must be removed at the end of each day's operation and a shower or bath taken.

(iv) Working clothes must be washed regularly, frequency depending on the toxicity of the pesticide used.

(v) Particular attention should be given to washing gloves, as contaminated gloves may be more dangerous than not wearing gloves at all.

(vi) Spraymen should clean themselves before eating.

(vii) Smoking, eating and drinking during spray operation must be strictly forbidden.

(viii) When work involves insecticides of relatively high toxicity, the hours of work must be arranged so that exposure to the material being used is not excessive; transport should be arranged so that there is not a long delay between the end of the days's operations and the return to the base for washing.

9.2.2.6. *Safety during preparation of spray material*

The greatest degree of exposure occurs during handling of the concentrates and facilities for their safe handling must be provided. When compounds of relatively high mammalian toxicity are to be used by non-commercial operators, these compounds should be supplied in diluted form.

In preparing concentrates of water-dispersible powders, use must be made of deep mixing vessels and long-handled mixers to protect the operator from splashing and to permit stirring from a standing position.

Long-handled dippers or scoops should be used for transferring the insecticide from one vessel to another. The concentrates may be subdivided into bags or small containers suitable for safe mixing by the spraymen in the field.

All small containers should be secured and packed to withstand transport to the periphery of the area of application.

Adequate protective clothing should be made available for those handling concentrates. Adequate washing facilities must be immediately accessible so that spills on the skin can be quickly removed.

The hazard of a pesticide in relation to the extent of exposure (contamination) can be reduced by:

1. Wearing appropriate protective clothing

2. Avoiding contact with insecticide to the minimum level and

3. Mastering the techniques of application.

9.2.3. **The period of exposure (Time)**

The exposure of spraymen to pesticides in confined areas, in residual spraying is among the highest in public health. Duration of work is one of the main factors influencing the extent of exposure. It is generally accepted that exposure should not exceed 5 hours a day and 5 - 6 days a week.

Therefore the hazard due to a pesticide in relation to the time of exposure can be reduced by:

1. Not exceeding prescribed working time for spraying

2. Washing contaminated skin during work and

3. Washing protective clothing frequently.

9.3. HAZARD TO PUBLIC AND SAFETY PRECAUTIONS

During residual spray operations, the inhabitants should be told the purpose and the times of insecticide applications and should be given clear instructions as to what they have to do before and after the treatment of their houses like removing foodstuff and cooking utensils, staying out of the house during spray operation and entering the house after the floors have been swept or washed, etc. The collected sweepings should be either burnt or buried well beneath the earth away from the poultry and cattle.

During space spray operations, there must be prior consultation with the health authorities responsible for

the residents. Special care must be taken to prevent the residents from re-entering the premises only after they have been adequately ventilated.

9.4. DISPOSALOF EMPTY OR NEAR EMPTY CONTAINERS

The containers must not be allowed to go astray or be removed by unauthorized people, who might use them as containers for storing food or drinking water especially in areas, where such containers are scarce. Used containers can be effectively decontaminated by rinsing two or three times with water, scrubbing the sides thoroughly. If a drum has contained an OP compound, an additional rinse with soda (5 %) should be carried out. The solution should be allowed to remain in the container overnight. Rubber gauntlets should be worn during the work and a soakage pit should be provided for disposing the rinsings. The emptied sachets and other toxic wastes should be burnt or buried.

9.5. INSECTICIDE RESIDUES IN THE ENVIRONMENT AND MAN

Stable pesticidal chemicals including their metabolic products and impurities can accumulate in the soil, water, grains, vegetables, fruits, oil-seeds and man. Following are some key-points that have become apparent from the extensive surveys on pesticide residues in man:

1. The chemicals found to accumulate in humans are mostly chlorinated hydrocarbons which are stable, lipophilic and are detectable at very low concentrations.

2. The levels of any given pesticide vary geographically and among various segments of the population.

3. The major factor determining the distribution of pesticides in the body is fat content; however, there are a number of other factors influencing the final distribution of residues.

Factors influencing the residue levels in man: Naturally factors such as food habits, physiological state and life style play important roles in determining the residue levels in man. The data from a residue survey of wild animals suggest that meat-eaters should accumulate more chlorinated hydrocarbon residues than vegetarians. The food habits of human vegetarians are not strictly comparable with those of herbivorous animals because human vegetarians consume relatively large quantities of oily food in our country. It has also been found that DDT residues are found more in the vegetarians than the meat-eaters in India. In short, only three factors of sex, race and age are known to be related to the final expression of residue levels among the nonoccupational general population groups within one country.

9.6.TREATMENT OF POISONING DUE TO PESTICIDES

Signs and symptoms of poisoning with dvfferent group of pesticides:

9.6.1.**With Organochlorines**: Generally poisoning from OC compounds is rare unless massive occupational exposure takes place. Within a few hours of ingestion or massive dermal exposure, the following symptoms due to CNS stimulation, headache along with dizziness followed by disorientation, vomitting, weakness of the skeletal muscles, tremors are noticed which finally lead to convulsions.

9.6.2.**With OP compounds**: Initial symptoms usually appear less than six hours after exposure to the insecticides. These symptoms include headache, weakness and fatigue. Affected people feel dizzy and their pupils sometimes become contracted; the patient may have trouble focusing his eyes. Abdominal cramps set in, accompanied by vomiting and diarrhoea, difficulty in breathing, profuse sweating and excessive salivation, sometimes followed by convulsions, coma and death.

9.6.3.**With carbamates**: Basically the symptoms due to carbamte poisoning are similar to that of a OP compound. The signs and symptoms due to a carbamate poisoning viz., headache, dizziness, nausea, vomiting, blurred vision, increased sweating, hypersalivation, tremor, fasciculations etc. appear sooner than with a OP compound.

9.6.4.**With pyrethrins and pyrethroids**: Symptoms with pyrethrin poisoning reflect the stimulation of the CNS. Some pyrethroids rarely cause eye irritation due to direct contact.

9.7. TREATMENT OF PESTICIDE POISONING:

Successful treatment of pesticide poisoning depends on the rapid and simultaneous application of measures for (a) alleviation of life-threatening effects, (b)removal of non-absorbed material and (c) symptomatic and/or specific treatment.

9.7.1.**The alleviation of life-threatening effects**: Use an oropharyngeal or nasopharyngeal airway or endotracehal intubation if airway obstruction persists. Artificial ventilation should be applied. Mouth-to-mouth respiration is to be avoided if the patient has been intoxicated by mouth because vomited material may contain dangerous amounts of toxic substances.

9.7.2.**The removal of non-absorbed mateial**: If clothing or exposed skin is contaminated by pesticide, the clothing must be removed and the skin washed with soap and water for atleast 10 minutes. Contamination of the eyes is treated by irrigation of the conjunctiva with water for 15 minutes.

9.7.3.**Symptomatic and/or specific treatment**:

9.7.3.1. *Intoxication with OP compounds*: Persons with or without signs of respiratory insufficiency should be treated with 2 - 4 mg of atropine sulfate and 1 - 2 g of a soluble salt of pralidoxime or 250 mg of obidoxime chloride by slow intravenous injection. In cases of severe intoxication, 4 - 6 mg of atropine sulfate should be given initially to adults followed by repeated doses of 2 mg or as much as is required to maintain full atropinization. Continuous intensive observation of patients is essential since symptoms may recur and therefore observation should be maintained for at least 72 hours after initial improvement.

9.7.3.2. *Intoxication with carbamates*: In case of accidental poisoning or manifest symptoms, 1 - 2 mg of atropine sulfate may be given intramuscularly or even intravenously. Care should be taken to avoid overdosage in cases of carbamate poisoning especially in children. Oximes should not be given.

9.7.3.3 *Intoxication with OC compounds*: Artificial ventilation should be made. Anticonvulsant treatment with soluble barbiturates, diazepam or paraldehyde should be given in sufficient dosage to calm the patient and prevent convulsions.

9.7.3.4. *Intoxication with anticoagulant rodenticides*: After taking blood samples for differential diagnostic tests including measurement of prothrombin levels, phytomenadione (vitamin K_1) in a dose of 5 - 10 mg should be given three times on the first day of treatment irrespective of symptoms. Prolonged observation of affected patients is required because these compounds are metabolized slowly and repititive therapy may be required.

9.8. FOR NEW COMPOUNDS:

9.8.1. Clinical examinations should be made by trained medical practitioner on spraymen before spray, immediately after spray, 24 hr, 3rd day and on 7th day after spray operation. The parameters to be observed are given in Annexure II. (Clinical Record)

9.8.2. Urine volume should be recorded and further analysis for metabolites should be observed.

9.8.3. Blood samples will be collected from all spraymen to determine **serum protein** and **Haematology** and also Bilirubin, Total protein, SGOT, SGPT, Alkaline phosphatase, Urea, Uric acid, Creatinine, Total Count and Differential Count.